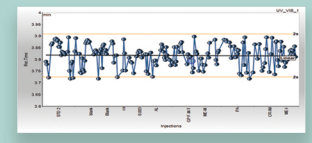
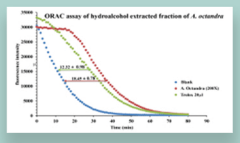
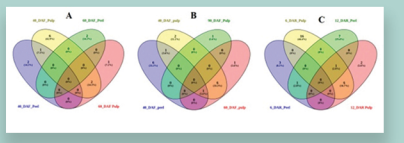
Regional Plant
Resource Centre
Bhubaneswar

RESEARCH & ACTIVITY REPORT 2022-23







RESEARCH AND ACTIVITY REPORT 2022-23

REGIONAL PLANT RESOURCE CENTRE • BHUBANESWAR

Research & Activity Report 2022-23

Published by
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Printed: January 2024

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Shri Pradip Kumar Amat
Minister
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Government of Odisha

MESSAGE

It is my pleasure to learn that RPRC, a reputed Research & Development Center, has been implementing various basic as well as applied research projects in the thrust area of plant taxonomy and conservation, biotechnology, biochemistry, microbiology, medicinal and aromatic plants and horticulture too. In addition to core research on plant biodiversity assessment, microbial applications, wild edible fruits, mangroves, orchids and phyto-chemicals of selected medicinal plants, few other aspects like production of economically important crops including tissue culture of banana, orchids are noteworthy as important thrust and frontier areas of plant sciences. It has consistently maintained and added to its unique collections of rare and endangered plants, orchids, bamboos, palms, cacti and succulents, mangroves, medicinal & aromatic plants, for developing a repository of bio-resources for research and conservation. I wish that the outcomes of the work carried out in RPRC would find its way to benefit all stakeholders of Odisha.

I wish that the Research & Activity Report (2022-23) published by the center would be useful for biodiversity conservationists, students, teachers and researchers as well.

(Pradip Kumar Amat)

Phyllanthus acidus (Narakoli)

Shri Satyabrata Sahu, IAS
Additional Chief Secretary
Forest, Environment & Climate Change Department
Government of Odisha

MESSAGE

As a Research & Development organization, under the Forest, Environment and Climate Change Department, Govt. of Odisha, the Regional Plant Resource Centre (RPRC), has been implementing several unique fundamental and applied research projects for wider use for conserving, propagating, and documenting the rich biological wealth of the region. Production of quality planting materials on commercial scale, germplasm conservation & analysis of wild edible fruits and re-introduction of rare and endangered plants including mangroves & orchids are some of the significant activities of the centre.

Recent initiative on development of Breeding & Tissue Culture facilities, Re-introduction of RET plants into field for conservation, is highly appreciable. I am glad to learn that RPRC has achieved Accreditation for Certification of T.C. Banana plantlets. I hope the Institute would continue to endeavor to find solutions to meet new challenges in conserving the biological diversity of the State.

I am hopeful that the findings of various research activities being undertaken by RPRC, highlighted in this Research & Activity Report (2022-23), would be of great help to various students, researchers and academicians.

(Satyabrata Sahu)

Carissa spinarun (Khira Koli)

FROM CHIEF EXECUTIVE'S DESK

Shri Suresh Pant, IFS
Chief Executive
Regional Plant Resource Centre

I am glad to bring out this Research & Activity Report 2022-23 which appraises implementation of various prioritized research programmes to address issues pertaining to conservation and bio-resource utilization relevant to the eastern ghats in general and Odisha state in particular. In continuation to our effort to establish germplasm banks of various plant groups for conservation and scientific enquiry, the center initiated and maintained research activities focusing the prioritized areas such as germplasm conservation and re-introduction of RET and other important special group of plants including mangroves and orchids, screening of wild edible fruits and medicinal plants for active bio-molecules, nutraceauticals, antioxidants, microbial applications (bioinoculant) for benefiting forest species and micro-propagation of plantation crops and endangered plants.

Every year, Scientists of RPRC are provided with research funds from state Forest, Environment & Climate Change Department under state plan budget after rigorous evaluation by the Research Advisory Committee (RAC) headed by the PCCF & HoFF in the Government. The centre has implemented several such research projects covering various thrust areas of research relevant to the sate as per recommendation of the RAC. Besides, Scientists are implementing research several research projects funded by external funding agencies like DBT, NMPB, RKVY, GoI etc. All the research activities & achievements are being reviewed & evaluated by Scientific Advisory Committee (SAC) at regular interval to guide & advice the scientists of RPRC in their scientific endeavours.

RPRC has been encouraging and nurturing academic intellect by guiding Ph.D. and M.Sc. students. A six month Project training progarmme for M.Sc. (Biotech) students from various organizations is being organized to provide hands on training to fulfill the requirement of their M.Sc. degree. Several research papers in national and international journals have been brought out by the Centre and many new processes and technologies have been developed.

All the research group, administrative group and supporting staff of RPRC made sustained effort and contributed to growth of the institute, and I extend my sincere thanks to them for their endeavour. Financial support received from various agencies of Govt. of India and Odisha is gratefully acknowledged. We are grateful to Additional Chief Seceretary, Forest, Environment and Climate Change Department, Government of Odisha for providing the research grant under state plan budget and support provided by Director, (Environment)-cum-Special Secretary is thankfully acknowledged.

(Suresh Pant)

Antidesma ghaesembilla (Nuniari)

1 | INTRODUCTION

Regional Plant Resource Centre (RPRC), Bhubaneswar, an autonomous R&D institute of Forest, Environment and Climate Change Department, Govt. of Odisha, has been taking all efforts to showcase its various R&D activities primarily through implementation of various research projects relevant to the state and establishment of different State of the Art facilities to produce and conserve plant genetic resources such as Modern Tissue Culture Laboratory, Orchidarium, Threatened Plants (RET) Garden: Wild Edible Fruits Garden, Medicinal Plants Garden, Cacti and other Succulents, Jagannath Vatika, Fragrant Flower Garden, Morning Health Walk etc. RPRC has implemented various research programmes under financial support from Forest, Environment & Climate Change Dept., Government of Odisha, RKVY, Science & Technology Dept., Govt. of Odisha, other apex central funding agency like DBT, NMPB, Govt. of India, which were prioritized to address issues pertaining to conservation and bio-resource utilization relevant to Odisha state.

During the year 2022-23, the Center initiated and maintained its core research activities focusing the prioritized areas such as re-introduction of RET and other important special group of plants including mang3roves and orchids, domestication and evaluation of wild edible fruits and medicinal plants for active bio-molecules, nutraceuticals, antioxidants, application of bioinoculant for forest species, useful secondary metabolites from fungi, micro-propagation of forest species, plantation crops and endangered plants. A total 3 external funded, 14 state plan funded projects have been implemented during the year 2022-23, engaged around 35 research fellows, published 28 research papers, 2 books, one Research & Activity Report (2021-22), trained 5 M.Sc. Biotech students for their PG degrees.

2 | MANDATE

The center has a mandate of promoting bioresource conservation, research and to augment plant resources for sustainable development in the following areas;

- Germplasm collection of selected plant groups (living collections) for long term conservation and research.
- Survey, evaluation, propagation and conservation of medicinal, aromatic, oil-yielding and other economic plants including rare/endangered species.
- Genetic manipulation of plants through cell, tissue and organ culture, somatic embryogenesis, transformation techniques and other biotechnological approaches.
- Studies on production, conversion and utilization of biomass especially of fuel-wood species.
- Provide necessary expertise and assistance in landscaping, garden lay out, green belt development, plant identification and impart training on plant propagation and nursery technologies.
- Dissemination of information through publication of scientific and popular articles.
- Co-operate and collaborate with other national and international institutions to promote the cause of conservation of biological diversity of plants and exchange of seed and plant materials.

3 | EXECUTIVE SUMMARY

Highlights of various research activities implimented during 2022-23 in Regional Plant Resource Centre are summarized below:

Harnessing the potential of endophytes of Piper longum as an alternate source for piperine production: optimization of protocol for laboratory production

The mass culture, extraction and chromatographic separation through siliqua column and comparison with standard revealed the presence of piperine by the selected fungal strain. In future the partially purified metabolite will be qualitatively and quantitatively analysed through HPLC, FTIR, HPTLC, GCMS.

Bioprocess optimization for enhanced recovery of L-glutaminase free L-asparaginase of fungal origin

Effect of different carbon and nitrogen sources required for L-asparagine production by selected fungal strains grown in solid and liquid phase of different incubation period have been studied. One selected fungal strain was experimented for mass culture, extraction, purification through salt precipitation, gel filtration and ion exchange chromatography. In future, purified enzyme will be detected through gel electrophoresis prior to evaluation for its anticancer bioactivity.

Morphotaxonomic characterization and documentation of fungi of Odisha

Detailed morphological description of species (alphabetically ordered) along with their plate culture photographs, microphotographs and camera lucida diagrams are provided. We are pleased to place this book as a part document on fungal diversity of Odisha to academicians and researchers. is an attempt to add taxonomic information along with the illustrated photographs and figures to encourage budding mycologists and biologists. This book will represent Odisha in Indian as well as Global context for Diversity and biology of fungi.

Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from Colletotrichum sp

Endophytes are diverse and ubiquitous group of microorganisms that resides in internal tissues of host plant without creating any harmful effects. Endophytic fungi have been reported as potential candidate for the production of various secondary metabolites and exploited in different agriculture, food, pharmaceutical and health care industries. The antimicrobial activity determined periodically with all separate solvent guided us to select DMSO and Ehanol as best separating solvent for this metabolite. Quantitative % inhibition revealed the DMSO and ethanol fraction for its positive bioactivity. The comparative data with antifungal metabolite revealed that the organism may produce antibiotic like compound. Present study revealed primary characterization of this fungus towards exploitable potential for bioactive compounds.

Establishment of Mass Propagation and Breeding Facilities for Orchids

As the climatic conditions in many parts of India are favourable for the proper growth and development of Orchids, all the procedures for producing planting materials and quality flowers are essential. Considering this, with the financialsupport of RKVY, one orchid cultivation and breeding facility centre has been developed which includes ***orchid cultivation,*** *orchid breeding,*

Standardization of Efficient Tissue Culture Based Propagation Methods for *Pomatocalpadecipiens* (Lindl.)J.J. Sm. and *Cymbidium bicolor*(Lindl.): Rare Orchids of Odisha

Propagation methods have been developed for two numbers wild orchids (*Cymbidium bicolor and Pomatocalpadecipiens*). Seeds of these orchids were collected from different natural habitats or from the orchidarium of Regional Plant Resource Centre and inoculated onMurashige and Skoog's (MS) medium for seed germination and seedling development.

Omics-approach to regulate ripening and enhance fruit shelf-life in banana: an important fruit crop for food security

Banana is a major staple food, which provides not only calories but also other important components for human nutrition such as vitamins, minerals, anti-oxidants and prebiotics. But excessive softening of the fruit leads towards the susceptibility of pathogens, which decreases the shelf-life of the fruit and results in huge post-harvest losses. Current investigation on the spatiotemporal expression of proteins in the peel and pulp tissue discloses a comprehensive information, and shed light on the key protein expression, tissue type and its involvement in different metabolic pathways of banana fruit during development and ripening. Through the genome editing or recent biotechnological approaches, functional role to the identified proteins can be assigned, thereby minimizing the post-harvest losses in banana due to the over ripening. It has been observed that varied spatiotemporal expression of the proteins that were regulating different metabolic pathways in banana peel and pulp tissues during developmental and ripening stages.

Developing efficient micro-propagation methods for some RET listed forest tree species of Odisha state plan project

P. santalinus L. , *L. glutinosa* (Lour) C.B ,*.L. comberi* Haines ,*P. simiarum*(Hook. F. &Thomson,. *Pterocarpus santalinus* are subjected to micropropagation to obtain tissue cultured plants to overcome uncertained process of seed germination.

Ameliorative effect of *Aporosa octandra* against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental rats.

The present study considers the bioassay of the extracts of its leaf to explore Ameliorative effect of *Aporosa octandra* against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental animal model. We have also considered the phytochemical investigation of the extracts

The protective diabetic neuropathy effect of *Buchanaia lanzan* Spreng. in streptozotocin induced type 2 diabetic rats.

The project was undertaken for exploring the therapeutic potential by evaluating the diabetic neuropathy effect of *Buchanania lanzan* Spreng (fruits) in Swiss albino rats. *Buchanania lanzan* Spreng. (Chironji/ Chara koli) (Anacardiaceae), 'Almondette tree' in English is distributed in tropical deciduous forests of North, Western and Central India mostly with a monsoonal climate. The phytoconstituent e.g., tannins, quercetin, gallic acid and glucoside were reported in bark of this plant. However, the traditional claim of *B. lanzan* as brain tonic to improve memory has not been scientifically validated yet. So, the project proposed to establish the protective diabetic neuropathy effect of *B. lanzan* in streptozotocin induced diabetic rats.

Development of Alternative regeneration method of rare mangrove species of *Xylocarpus* through vegetative propagation

The artificial regeneration of rare, endangered, and threatened species like *Xylocarpus* through vegetative methods has been a challenging effort. As an alternative method of seed propagation or natural regeneration , vegetative propagation methods were attempted for the mangrove plants *Xylocarpus granatum, Xylocarpus mekongensis & Xylocarpus moluccensis* . Alternative methods i.e.air- layering found effective in *X. granatum* and *X. mekongensis* as far as rooting & sapling establishment are concerned. However, *X. moluccensis* did not respond in artificial rooting and acted as difficult-to-root species. For re-introduction, further mass propagation and hardening of saplings are in progress.

Immunity Boosting Natural Fruits: Determination of Ascorbic Acid (Vitamin-C) and Other Antioxidants For Selection of Potent Species To Promote Domestication.

Wild edible fruits are considered as nutraceuticals since they offer both nutritional and potential therapeutic

benefits..Many studies have revealed that tropical fruits with high levels of vitamin C include lemons, oranges, jackfruit, guava, etc. However, reports suggest that low- and middle-income country citizens are deficient in vitamin C. This could be reduced by encouraging people to eat natural fruits with ascorbic acid levels close to those of cultivated fruits rather than taking supplements because natural fruits also contain various antioxidants in addition to vitamin C. Thus, it is now more important than ever to domesticate natural fruits as immune boosters on a big scale.In this context, the following objectives are taken into consideration for the study i.e. Isolation and quantification of Ascorbic acid (vitamin C) and other antioxidant properties in selected natural fruits in order to select the potent species to encourage its conservation and domestication as immune boosting natural fruits.

Field introduction and establishment studies of variously propagated wild edible fruits of Odisha

Conservation of wild edible fruit species is equally important as it helps to safeguard the genetic diversity of these species. The wild edible fruits of Odisha are a significant natural resource that provides valuable nutrition and income for local populations. However, it is essential to conserve them and promote their sustainable use to ensure their continued availability for future generations. During the 1st year of this project, site selection has been initiated (Chandaka Wildlife Division) for 1st phase introduction of 6 wild edible fruit species. However, the re-introduction work will be done in more other suitable sites as a part of continuation of this project work to obtain comparative data on growth and development of the introduced species to mark adaptability.

Assessment of antifungal activity of *Combretum roxburghii* and *Terminalia arjuna* solvent extracts against *Aspergillus flavus*.

In this project, Antifungal activity using radical growth method, Antifungal activity using agar well diffusion method,Isolation of aflatoxin from liquid medium and anti aflatoxin activity of solvent extracts, Antifungal activity using agar diffusion method, Effect of extracts on biomass of fungus using liquid broth medium and aflatoxin isolation have been performed.

Evaluation of non viable seeds of *Withania somnifera* for biological activity.

Withania somnifera is a cultivated crop, its seeds loose viability very quickly at room temperature. In the present study biological potential of non viable seeds was explored so as to put them for use in medicinal purpose.

Propagation and reintroduction of selected endangered species of Odisha

Seed germination experiments with of *Oroxylum indicum* ,*Pterocarpus santalinus* , *Cryptocarya amygdalina Alphonsea madraspatana*, *Litsea glutinosa* and *Cordia macleodii were* attempted and advised that alternative methods for mass multiplication must be pursued.

Standardization of *in vitro* regeneration techniques in red banana and establishment of red banana in Odisha climate condition

Tissue culture banana or micropropagation of banana is an important and sigificant technique used for the large-scale production of banana plants. It involves the propagation of plants using tissue culture techniques, allowing for the mass production of disease-free and genetically identical plants. One of the main benefits of tissue culture banana is the ability to rapidly prodcue large numbers of healthy plants with predictable characteristics. This method also allows for the production of plants that are resistant to diseases and pests, which is crucial in the banana industry since bananas are highly susceptible to various diseases. It is also important for the preservation of banana biodiversity. The banana industry has a limited number of commercially important cultivars and tissue culture techniques allow for the preservation and propagation of rare or endangered cultivars. In addition to its commercial benefits, tissue culture banana is also valuable for research purposes. It allows for the study of plant growth and development in a controlled environment, which can lead to a better understanding of plant physiology and genetics.

4 | RESEARCH ACHIEVEMENTS

4.1 Harnessing the potential of endophytes of Piper longum as an alternate source for piperine production: optimization of protocol for laboratory production

Principal Investigator : Dr. Nibha Gupta, Principal Scientist

Piper longum L., a plant belonging to the Piperaceae family, is recognized for its production of diverse bioactive secondary metabolites such as alkaloid Piperine. Several endophytic fungi isolated from this host plant have previously been documented for their potential to produce similar bioactive compounds with a broad spectrum of biological properties including antimicrobial, antioxidant, anti-inflammatory, antidepressant and anticancer activities.A few reports are available on production of piperine from endophytic fungi.But no reports were available on optimization of culture media and evaluation of anticancer activities. The main objective of this study was to optimize the culture condition for better production of Piperine in laboratory condition where 37 nos. of fungal isolates have been screened for piperine production. Four fungal strains were selected for the enhancement in metabolite production under different nutritional conditions, pH and incubation period. Out of 37nos. of fungal isolates, 4 nos. of fungal strains F1, F2,F3,F4 were selected in an incubation period of 7 days with pH 5.5. In this condition, fungal strain F1 was giving better yield. Then screening of different nutritional conditions such as effect of different pH, carbon source, nitrogen source on F1 was carried out. Results revealed that lactose, peptone and amino acid with pH 6.5 was giving better yield as compared to normal condition. The mass culture , extraction and chromatographic separation through siliqua column and comparison with standard revealed the presence of piperine by the selected fungal strain. In future the partially purified metabolite will be qualitatively and quantitatively analysed through HPLC, FTIR, HPTLC, GCMS.

4.2 Bioprocess optimization for enhanced recovery of L-glutaminase free L-asparaginase of fungal origin

Principal Investigator : Dr. Nibha Gupta, Principal Scientist

All enzymes are proteins, catalyze different physiological and metabolic reactions in normal endowed growth. Many enzymes from plants, animals and microbial origin have been reported for their useful extracellular activity. Several of them have been explored for different industrial, agricultural and health care product design and formulation among them. L-asparaginase is an important amino acid degrading enzyme being studied continuously as an health care agent especially for treatment of acute lymphoblastic and Non Hodgkin lymphoma and melanosarcoma as an effective antitumor agent. L-asparaginase (L-ASNase, also called L-asparagine amidohydrolase with Enzyme Commission number 3.5.1.1) is an enzyme involved in the catalysis of the conversion of L-asparagine in its free amino acid form to L-aspartate and ammonia. The present work aimed to study the impact of different carbon and nitrogen sources on the effective production of fungal strains of glutaminase free L-asparaginase activity. current work tested 260 fungal strains. Isolated fungi were tested using conventional plate assay method with indicator dye, phenol red. L-asparaginase activity was measured by cultivating in modified czapek Dox medium. 4 fungal strainsi.e. (F1, F2, F3, F4) have shown positive result for L-glutaminase free L-asparaginase activity. The present study was carried out using two organisms i.e. (F3, F4) to test the potentiality of fungi for L-asparaginase, L-glutaminase and Urease production by executing experimentations. Fungal strainF3was having L-aspraginase, L-glutaminase and Urease activity in phenolred plate test method but in case of Bromothymol blue (BTB) method, it showed glutaminase free of L-asparaginase but urease showed positive result. In case offungal strain F4, phenol red method showed glutaminase free of L-asparaginase but positive result in case of urease activity. Phenolred dye test can give better result in the effect of carbon and nitrogen sources on glutaminase free L-asparaginase enzyme activity as compared to BTB dye test in case of both the fungi. Effect of different carbon and nitrogen sources required for L-asparagine production by selected fungal strains grown in solid and liquid phase of different incubation period have

been studied. One selected fungal strain was experimented for mass culture, extraction, purification through salt precipitation, gel filtration and ion exchange chromatography. In future, purified enzyme will be detected through gel electrophoresis prior to evaluation for its anticancer bioactivity.

4.3 Morphotaxonomic characterization and documentation of fungi of Odisha

Principal Investigator : Dr. Nibha Gupta, Principal Scientist

Fungi are important group of eucarytotic microorganisms and diversified pertaining to its morphology, physiological and metabolic activity. Besides association with other living organisms in the world, environmental and edaphic factors undoubtedly reveal vital role in creating diversity among them. Odisha endowed with wealth of diversified resources including heavy metal mine, mangroves, good and huge forest cover, very long sea coasts which develop a most suitable habitat for fungi and attained opportunity for multifarious diversity. We have described >200 no. of fungal isolates through plate culture and slide culture techniques to characterize the morphology and colony features (such as shape, size, color, elevation, margin, surface texture, ornamentation, sprorulation, exudates, and pigment production, among others. The sporulation characteristics of fungus were observed using the slide culture method. We were able to view undisturbed and attached sporophores and facilitate identification by using this method. Fungal sporulation patterns were watched, measured, and microphotographed using a Nikon 50i and sketched camera lucida drawings and identified. The data recorded for morphotaxonomic observations were systematically placed Part I and II of Fungi of Odisha. The content mainly deals with morphotaxonomic descriptions of fungi isolated differently, preserved & maintained in the Microbiology laboratory of RPRC, Bhubaneswar, Odisha. Dedication and devotion of two years endowed towards this work come out with documentation of >200 no. of fungi from Odisha as part I and II. Detailed morphological description of species (alphabetically ordered) along with their plate culture photographs, microphotographs and camera lucida diagrams are provided. We are pleased to place this book as a part document on fungal diversity of Odisha to academicians and researchers. is an attempt to add taxonomic information along with the illustrated photographs and figures to encourage budding mycologists and biologists. This book will represent Odisha in Indian as well as Global context for Diversity and biology of fungi.

4.4 Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from Colletotrichum sp

Principal Investigator : Dr. Nibha Gupta, Principal Scientist

Endophytes are diverse and ubiquitous group of microorganisms that resides in internal tissues of host plant without creating any harmful effects. Endophytic fungi have been reported as potential candidate for the production of various secondary metabolites and exploited in different agriculture, food, pharmaceutical and health care industries (Aharwal et al., 2016). Sometimes, they have biosynthetic potential in order to produce similar kind of metabolites as synthesized and produced by host plants. Such types of adaptive traits, they acquire as a result of long term association with their host plants. In short, they can mimic the specific secondary metabolism and produce the same or similar kind of metabolite as their host plant does. On the other hand the huge requirement of potent drugs for various diseases induced researchers to explore and investigate alternative avenues for searching novel bioactive molecules. Present work deals with extraction, purification and characterization of secondary metabolite from an endophytic fungus .The fungal culture was grown in basic nutrient medium for 21 days at 30 C. Ethyl acetate fractionation at room temperature at the rate o 1:2 ratio followed by subsequent drying and dissolution in methanol secured metabolites. This was subjected to colomn chromatography prepared with Siliqua and eluted with admixture of different solvents, fractions were pooled , dried and crystallized. The pooled samples were eluted with different solvents polar to non polar. The antimicrobial activity determined periodically with all separate solvent guided us to select DMSO and Ehanol as best separating solvent for this metabolite. Quantitative % inhibition revealed the DMSO and ethanol fraction

for its positive bioactivity. The comparative data with antifungal metabolite revealed that the organism may produce antibiotic like compound. Present study revealed primary characterization of this fungus towards exploitable potential for bioactive compounds.

4.5 Establishment of Mass Propagation and Breeding Facilities for Orchids

Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist

1.Establishment of orchid cultivation and breeding facility

Orchids are mostly used for their high ornamental value; they are mostly used in potted conditions or as cut flowers. Under natural conditions,more than 25000 species have been reported and using these, more than 1 lakh hybrids have been developed to meet the market demand. However, there are tremendous scopes for the development of new combinations. Business on orchids is increasing in every corner of the world, and theprimary production centres arein Asian countries. As the climatic conditions in many parts of India are favourable for the proper growth and development of Orchids, all the procedures for producing planting materials and quality flowers are essential. Considering this, with the financialsupport of RKVY, one orchid cultivation and breeding facility centre has been developed (Fig1).

Fig.1. Inauguration of the Orchid Cultivation and Breeding Facility by Smt. Mona Sharma, Additional Chief Secretary, Forest, Environment and Climate Change Department, Govt. of Odisha.

The orchid cultivation facility

The facility has been developed to standardize the cultivation practices for the Dendrobium orchids widely used for cut-flower production. The plants are growing under protected conditions inside the polyhouses. The polyhouse is equipped with a top ventilation system, sliding shadenets to control the light intensity, sprinklers for irrigation and forgers for managing the humidity and temperature. Double-door entry is provided to prevent the entrance of the insects. Inside the polyhouses, raised shade net beds are developed for the proper growth and development of the epiphytic orchids. On the shadenet beds, coconut husks are kept on which the plants are growing. Three polyhouses have been developed with a capacity of growing 6,800 plants. The following varieties are growing in these facilities (Fig 2).

Fig. 2 Orchid cultivation facility : Cultivation chamber of *Dendrobium* Singapore White and *Dendrobium* Sanun White; b & c) cultivation chamber of *Dendrobium* Ersakul; d) Inside view of *Dendrobium* Ersakul cultivation.

Dendrobium Ersakul

Fig.3. Features of *Dendrobium* Ersakul: The plants with flowers growing on a pot, b) View of an inflorescence, c&d) Close views of the flowers.

This variety is a hybrid orchid developed for cut-flower production and used worldwide. The important feature of the variety is that it produces highly attractive flowers with a unique shape, size and bright purple colour. The average lengths of the plants are 10-15cm inch, with a stem diameter of 2-3 cm and able to produce five to seven inflorescence in a year. The average length of the inflorescences is 40-50cm and contains 8-10 numbers of flowers. The flowers' unique feature is that they can stay more than a month under appropriate environmental conditions. The tropical environmental conditions are ideal for the proper growth of this variety (Fig 3).

Dendrobium Sanun White

Fig.4. Features of *Dendrobium* Sanun White :a) The plants with flowers growing on a coconut husk, b) View of an inflorescence, c &d) Close views of the flowers.

This white-coloured flower variety is widely used for different arrangement purposes, excellent flowers to be arranged with other flowers. Demands of the flowers are amazing, thus used extensively throughout the world. The cultivation of this variety also has been adopted by the state's farmers. Similar to *Dendrobium*Ersakul, this variety can also be grown under similar conditions on the coconut husk pieces. The plants under cultivation conditions can reach up to 20-30cm. In general, the stem diameter ranged between 1.5 to 2.0 cm and each stem used to have 8-10 numbers of leaves arranged alternatively. The major attraction of the variety isthat it is easy to cultivate under protected cultivation and produces flower profusely throughout the year. From each plant, 5-7 inflorescence can be produced, and each inflorescence is 30-40 cm long, having 8-10 flowers on average. After cutting from the plants, the flowers can be kept for one month under appropriate conditions. Tropical climatic conditions are ideal for proper growth and development (Fig 4).

Dendrobium Singapore White

Fig. 5.Features of *Dendrobium*Singapore White: a)The plants with flowers growing on coconut husk b) View of an inflorescence, c &d) Close views of the flowers.

This hybrid orchid is widely used for cut-flower production, popular because of its unique flower shape and bright white in colour. In any flower arrangement, this can be used alone or in combination with other flowers. Whenever only white colour flowers are used for any specialized function, this variety is most suitable. As of the different cane-type *Dendrobium* varieties, these can also be grown on the coconut husk under protected conditions. Under polyhouse conditions, the plants can reach a height of 20 to 30 cm, containing10-15 numbers of leaves. From each plant,4.5 inflorescences can be harvested within a year, and each inflorescence will have 8-10numbers of flowers. The self-life of the flowers used to have 30 days on the plants under appropriate conditions. In general, tropical conditions are ideal for proper growth and development (Fig 5).

The flowers of these are harvested around the year based on the requirement of the market, currently, each flower costs Rs. 15/pc among the three varieties. The best quality of the flower stick used to have 8-10 flowers of which 3-4 should be in the budding stage. These are being cut from the plants and bundled to supply into the local market. From the facility,3945 flowers have been harvested and supplied to the market in the first year (Fig 6).

Fig.6. Harvesting of the flowers: a. Collection after harvest, b & c Flowers are sold in bundles.

Evaluation of growth and development of three different cultivars of *Dendrobium* orchid.

Three different cultivators, *Dendrobium* Ersakul, *Dendrobium* Singapore White and *Dendrobium* Sanun White were used for this study. Tissue culture raised plants of these were planted on coconut husks and grown under specific conditions of light, temperature and humidity inside polyhouses. The spaces between the plants were of 30 cm for all the varieties. After planting, appropriate irrigation and fertilizers were applied, and their growth and development were monitored at different intervals. Details of the observations after 12 months of cultivation are mentioned in the (Table1).

Table 1- Plant Growth parameters of Dendrobium Varieties after 12 months of cultivation

Sl. No.	Name of the Variety	Plant Heightin cm.	No. of Stems	No. of Leaves	Flower per Inflorescence	Inflorescence height in cm.	Flower Length in cm	Flower width in cm
1	*Dendrobium* Ersakul	12.50	8.00	20.00	14.00	54.00	8.40	8.40
2	*Dendrobium* Singapore white	24.50	5.00	12.00	12.00	47.00	6.20	6.40
3	*Dendrobium* Sanun white	24.50	6.00	14.00	14.00	47.50	7.10	7.40

Observationswere made from five plants from each variety.

From the observation, it was found that the plants of*Dendrobium* Ersakul cultivars, most widely used for cut-flower production, reached up to the height of 12.50 cm and lower than that of the other two cultivars those reached up to the height of 24.50 cm at 12 months of cultivation (Table 1).However, the *Dendrobium* Ersakul produces more numbers of leaves and stems as compared to that of the white varieties. In all three varieties,the inflorescence length could reach up to 45 cm, and each hadmore than 12 flowers per inflorescence. Aboutthe flower size, it was found that the *Dendrobium* Ersakul produces bigger flowers as compared to others (Table 1).

Establishment of the orchid breeding facility

The facility was established with an aim to develop new orchid hybrids to meet the market demand. In India, more than 1300 species have been reported,the *Dendrobium* orchids do have tremendous demand to be used for breeding purposes. In the state of Odisha,11 species of *Dendrobium* are growing in different natural habitats, and all of these could be used to develop new varieties. For cut flower production, the most important feature is the self-life of the flowers; the wild orchids could be used for this purpose. Other features like new flower colour, shape and size could be developed. Similarly, different features of *Phalaenopsis*, *Vanda*, *Cattleya*, *Cymbidium*and other orchids could be developed.

Taking these above features, wide arranges of orchid germplasms have been collected,and their cultivation condition have been standardized. Details of the germplasmsavailable in the orchid breeding centre are mentioned in Table 2.

Table 2. Germplasm collections of orchids for the orchid breeding centre.

SI No.	Name of Orchid	No of Species/Varieites
1	*Dendrobium*	122
2	*Cattleya*	23
3	*Phalaenopsis*	30
4	*Vanda*	65
5	*Mokara*	4
6	*Aranda*	15
7	*Spathoglottis*	2
8	*Cymbidium*	16
9	*Aerides*	6
10	*Oncidium*	5
11	*Paphiopedilum*	2

The flowers of these are harvested around the year based on the requirement of the market, currently, each flower costs Rs. 15/pc among the three varieties. The best quality of the flower stick used to have 8-10 flowers of which 3-4 should be in the budding stage. These are being cut from the plants and bundled to supply into the local market. From the facility,3945 flowers have been harvested and supplied to the market in the first year (Fig 6).

Propagation of orchids through tissue culture

Tissue culture is the best method for the propagation of orchids. As there istremendous demand for the planting materials for *Dendrobium* orchids, procedures have been developed for the eleven different hybrids (Fig. 7). The seeds of the orchids lack endosperms and, thus, do not germinate easily under natural conditions, however, under tissue culture condition whenever nutrients are supplied,more than 90 % of the seeds able to complete germination. In this study, the seeds were formed with hand pollination on the plants growing in the greenhouses of RPRC and were allowed to reach the mature stage. After surface sterilization, the seeds were inoculated on the Murashige and Skoog's (MS) medium and then cultured under 16L/8D light/dark at 25°C. The MS medium was supplemented with Benzylaminopurine (BAP) 0.5 mg/l for enhancing the seed germination process. All the varieties completed the seed germination process at 30 days of the culture, and the germination

rate was above 82%. The seeds germinated after 15 days of inoculation, and protocorms were formed at 45 days of culture. The leaf and roots were initiated at 90days of culture. Whenever the plants reached the height of 1 cm, young shoots were transferred to MS medium containing 2.5 mg/l BAP to produce new shoots. It has been observed that nearly 25 new shoots were produced from each explant at 120 days of culture. Following this procedure, 1000s of new shoots could be produced in a short time. The shoots produced on the BAP medium were then transferred to MS medium containing Indolebutyricacid (IBA) for shoot elongation and root development. On transferring to this medium,the plants reached to a height of 6 to 8 cm after 180 days of culture, at this stage, each shoot develops 4-5 leaves and 2-3 roots (Fig. 8). The young seedlings were then transferred to the coconut husk pieces and kept on the polyhouses under appropriate conditions required for orchid growth and development (Fig. 9). The acclimatized seedlings were then transferred to the pots for further growth and flower development.

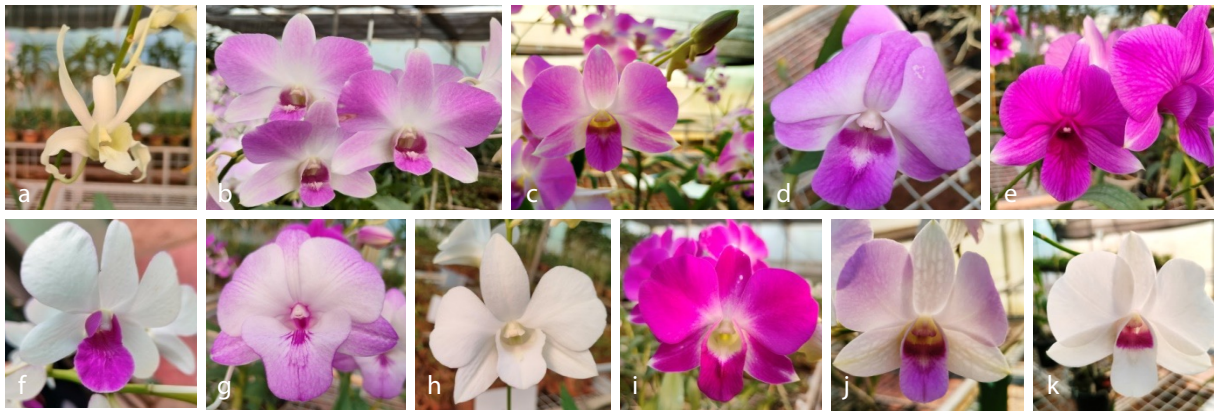


Fig.7. *Dendrobium* orchid varieties used for the mass propagation through tissue culture.

a. *Dendrobium Fairy White, b. Dendrobium Light Pink, c. Dendrobium Aridang Blue, d. Dendrobium Burana Jade, e. Dendrobium Charak Red, f. Dendrobium White Beauty, g. Dendrobium Emma White Mutation, h. Dendrobium Compact White, i. Dendrobium Aridang Pink, j. Dendrobium Pink Fragrance, k. Dendrobium Buta Chung*

Fig.8. In vitro propagation of different varieties of *Dendrobium*

Fig. 9.Different stages of growth and development of *Dendrobium* orchids through tissue culture.

a) Primary hardening of the tissue culture raised plants on coconut husk pieces.,b) Secondary hardening of the tissue culture raised plants on pots containing orchid potting media.c)Flower development on the tissue culture raised plants.

Propagation of wild orchids through tissue culture.

From the state of Odisha, 151 orchids have been reported from the different natural habitats, and among these, eight species are endemic to the state.All the orchids are considered to be rare, and extensive conservation measures need to be taken. Ofthe many procedures, mass-scale propagation and introduction into the natural habitats is the most viable method. Taking in this view, during this year, propagation methods have been developed for seven numbers wild orchids (*Aerides odorata, Aerides multiflora, Acampe praemorsa, Cymbidium bicolor, Pomatocalpa decipiens and Vanda tessellate*). Seeds of these orchids were collected from different natural habitats or from the orchidarium ofRegional Plant Resource Centre and inoculated on Murashige and Skoog's (MS) medium for seed germination and seedling development. The effects of different growth regulators on the development of seedlings were also evaluated. Besides,vegetative parts like young shoot buds developed in vitro were cultured on MS medium containing different growth regulators for the production of the new plants of the wild orchids.

4.6 Standardization of Efficient Tissue Culture Based Propagation Methods for Pomatocalpa decipiens (Lindl.)J.J. Sm. and Cymbidium bicolor(Lindl.): Rare Orchids of Odisha

Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist

From the state of Odisha, 151 orchids have been reported from the different natural habitats, of these eight species are endemic to the state. All the orchids are considered to be rare and extensive conservation measures needs to be taken. Of the many procedures, propagation in mass scale and introduction into the natural habitats is the most viable method. Taking in this view, during this year, propagation methods have been developed for two numbers wild orchids (Cymbidium bicolor and Pomatocalpadecipiens). Seeds of these orchids were collected from different natural habitats or from the orchidarium of Regional Plant Resource Centre and inoculated onMurashige and Skoog's (MS) medium for seed germination and seedling development. Effects of different growth regulators on development of seedlings also evaluated. Besides, vegetative parts like, young shoot buds developed in vitro were cultured on MS medium containing different growth regulators for production of the new plants of the wild orchids.

4.7 Omics-approach to regulate ripening and enhance fruit shelf-life in banana: an important fruit crop for food security

Principal Investigator: Dr.Giridara Kumar Surabhi, Senior Scientist

Banana is a major staple food, which provides not only calories but also other important components for human nutrition such as vitamins, minerals, anti-oxidants and prebiotics. It is a fruit crop of global importance with an annual production of 102 million tonnes worldwide (http://faostat.fao.org).It is a typical climacteric fruit that follows ethylene dependent ripening, that regulates a series of ripening associated processes such as climacteric respiration, peel de-greening and pulp softening. However, excessive softening of the fruit leads towards the susceptibility of pathogens, which decreases the shelf-life of the fruit and results in huge post-harvest losses.

Till date most of the fruit proteome studies were restricted to single tissue type and limited number of development and ripening stages, whereas the metabolic pathways related to protein and their abundance during fruit development and ripening is still far from fruition. In this regard, the current study performed the first spatiotemporal expression of the proteins involved in diverse metabolic pathways, during different stages of fruit development and ripening in bananas. The present study provides an important insight into the molecular networks underlying banana fruit development and ripening. The objective of the study is (i) to decipher the proteome dynamic changes in spatiotemporal manner in peel and pulp tissue of banana using high-throughput proteomics platform, (ii) to gain molecular insights of the proteins in relevance to different metabolic pathways and their interactions in banana peel and pulp tissues, during different developmental and ripening stages. Our study highlights the extensive peptide coverage and also resulted in the identification of tissue specific proteins that are involved in different biological processes and metabolic pathways during banana development and ripening. Identified proteins could be highly useful in conducting further investigation to assign individual functional roles in fruit ripening and shelf-life in bananas, thereby reducing post-harvest losses.

Many researchers have focused on identifying differentially expressed proteins during fruit ripening, though the sampling was limited to a specific stage and single tissue type. However, proteins involved in different metabolic pathways that are operational during early fruit development to maturation and ripening remain unexplored to date to the best of our knowledge. In this regard, current investigation on the spatiotemporal expression of proteins in the peel and pulp tissue discloses a comprehensive information, and shed light on the key protein expression, tissue type and its involvement in different metabolic pathways of banana fruit during development and ripening. To our knowledge, this is the first in-depth proteome study which is focused on spatiotemporal expression of protein dynamics involved in different metabolic pathways in banana fruit. The result from our study not only provides an insight of the protein changes during banana development and ripening process, but also enlightens the path for further utilization of these identified proteins for banana crop improvement programs.

High abundance of ethylene biosynthesis related protein namely SAM synthase was observed in 12-DAR peel during ripening, suggesting that the ethylene may play an active role in fruit ripening. Proteins involved in hormone regulation such as ARF-GAP and TPR_domain containing protein related to auxin and ABA biosynthesis exhibited high abundance during banana ripening, suggests that the auxin and ABA may have a key role in promoting fruit ripening, other than ethylene in banana. XTH which is considered as a major cell wall modification protein, displayed an increase in the protein expression in peel tissue during later stage of banana ripening i.e., 12-DAR peel, which suggests that the XTH proteins actively participates in the regulation

of cell wall modification process during peel ripening. Further, some of the unique or distinct proteins were identified in specific stage of banana during development and ripening. Protein-protein interaction revealed that two number of glycolysis pathway related proteins namely, PGM and enolase found to be interacting more with other protein partners in the network. Through the genome editing or recent biotechnological approaches, functional role to the identified proteins can be assigned, thereby minimizing the post-harvest losses in banana due to the over ripening.

We observed varied spatiotemporal expression of the proteins that were regulating different metabolic pathways in banana peel and pulp tissues during developmental and ripening stages(Fig. 7A-J). An increase in the expression pattern of α-man was observed in the pulp tissue from 40-DAF to 6-DAR (4-fold), but sharply decreased at 12-DAR pulp. Whereas, in case of peel tissue, the expression pattern of α-Man protein gradually increased from 40- to 90-DAF, but decreased in the later stages of ripening i.e., 6- and 12-DAR (Fig. 7A). β-gal protein showed an increase in the abundance in both peel and pulp at 40-, 60- and 90-DAF, but decreased at 6-DAR peel and pulp. Whereas, same protein expressed at 12-DAR peel but decreased expression at 12-DAR pulp (Fig. 7B).

Our result showed that XTH protein which is involved in cell wall modification were found to be more abundant in peel tissue of 12-DAR (3-fold) than other developmental stages i.e., 40, 60 and 90-DAF and 6-DAR. Whereas, in the case of pulp, the pattern of XTH gradually increased at developmental stages (40 to 90-DAF), but slightly decreased at 6 and 12-DAR (Fig. 7D). The abundance of the PE proteins in peel showed declined at 60-DAF, and sharply increased at 90-DAF, 6 and 12-DAR. In the case of pulp, the expression of PE proteins was found to decrease from 40-DAF to 6-DAR, but a sharp increase in the pattern was observed at 12-DAR (Fig. 7C).

Starch biosynthesis related protein such as starch synthase showed an increase (2-fold) in the abundance in both peel and pulp at the initial stage of fruit development i.e., 40-DAF, but decreased with progression of banana ripening (Fig. 7E). Glucose-1-phosphate adenylyltransferase protein which is also related to starch biosynthesis were found to be increased in both peel and pulp during developmental stages of banana i.e., 40,60 and 90-DAF, but decreased during ripening stages i.e., 6 and 12-DAR (Fig. 7F).

Sugar metabolism related proteins such as sucrose synthase and fructose-bis-phosphate aldolase showed an increase in the abundance in pulp during ripening stage i.e., 12-DAR. The expression pattern of sucrose synthase and fructose-bisphosphate aldolase proteins in pulp gradually increased from 40-DAF to 12-DAR, but slightly decreased at 12-DAR peel (Fig. 7G, H).

S-adenosylmethionine synthase (SAM-synthase) proteins which are known to be involved in ethylene biosynthesis was gradually increased in both peel and pulp from 40-DAF to 6-DAR, but later showed declined in the expression in 12-DAR pulp. Highest expression of SAM synthase protein was observed in 12-DAR peel (3-fold) (Fig. 7I).

Identified proteins (presented in bold letter) known to regulate different metabolic pathways during banana fruit development and ripening process were represented schematically (Fig. 8). Interestingly, transcription factors related proteins i.e., bZIP, NAC-A/B and AP2/ERF are known to regulate different metabolic pathways such as hormone regulation and sugar metabolism which resulted in cell wall loosening during ripening. Ethylene suggested to be cross talk with other phytohormones such as auxin and IBA for triggering the fruit ripening process (Fig. 8). In contrast, some of the proteins were found to be present at specific stage and tissue type of banana, which were represented in the numerical format.

Venn diagrams representing the common and distinct proteins through various comparisons (A) 40-DAF pulp, 60-DAF peel, 40-DAF peel and 60-DAF pulp, (B) 40-DAF pulp, 90-DAF pulp, 40-DAF peel and 60-DAF pulp, (C) 6-DAR pulp, 12-DAR peel, 6-DAR peel and 12-DAR pulp depicted in Fig. 9A, C. In case of 40-DAF pulp and 60-DAF peel, a total of 6 (42.9%) and 2 (14.3%) unique proteins, with no common proteins. A total of 2 (14.3%) and 1 (7.1%) distinct protein, without having overlapping proteins were identified in 40-DAF peel and 60-DAF pulp, respectively. Further, a total of 2 (11.1%) and 1 (5.6%) protein were unique for 40-DAF pulp and 90-DAF pulp. Six (33.3%) and 1 (5.6%) distinct protein, without sharing any overlapping proteins were identified in 40-DAF peel and 60-DAF pulp, respectively. In case of 6-DAR pulp and 12-DAR peel, a total of 16 (44.4%) and 7 (19.4%) distinct protein were present without sharing any common proteins. Likewise, unique proteins of 3 (8.3%) and 2 (5.6%) were present in 6-DAR peel and 12-DAR pulp, suggesting the presence of distinct unique proteins may have a regulatory role at specific stage and tissue type.

Fig. 10, presents the protein-protein interaction network analysis of peel and pulp tissues of different developmental and ripening stages by using geneMania. Two number of glycolysis related proteins i.e., enolase and PGM proteins were found to be interacting more with other proteins. PGM acts an important regulatory enzyme in cellular glucose utilization and energy homeostasis process during ripening and also it participates in sugar metabolism. Whereas, enolase is involved in the energy metabolism and carbon flow process during fruit ripening.

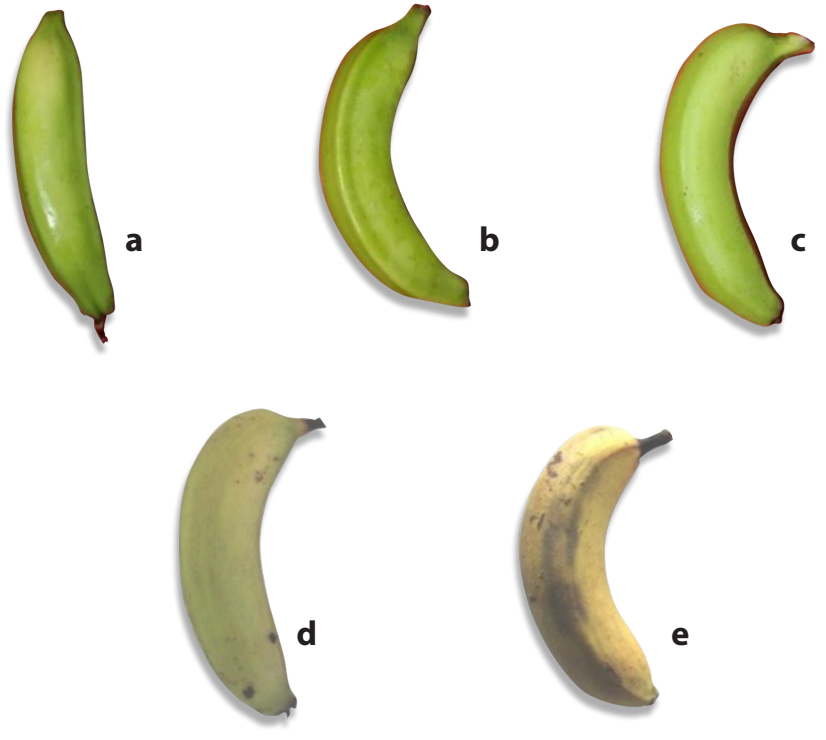


Fig. 1: Visual appearance of banana cv. Grand Naine at different developmental and ripening stages. Fruit sampling was done at 40-DAF**(A)**, 60-DAF**(B)**, 90-DAF **(C)**, 6-DAR **(D)**, 12-DAR **(E)** and considered for the biometric, biochemical and proteome analysis.

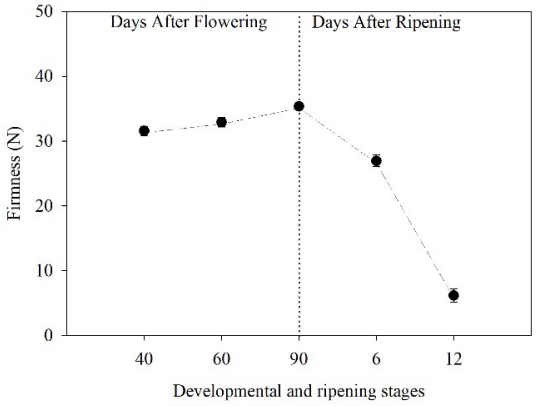


Fig.2: Pulp firmness at different developmental and ripening stages. Each valuerepresents the mean of three biological replications of three fruits analyzed at each ripening stage and vertical error bars represent the standard deviation (±SD).

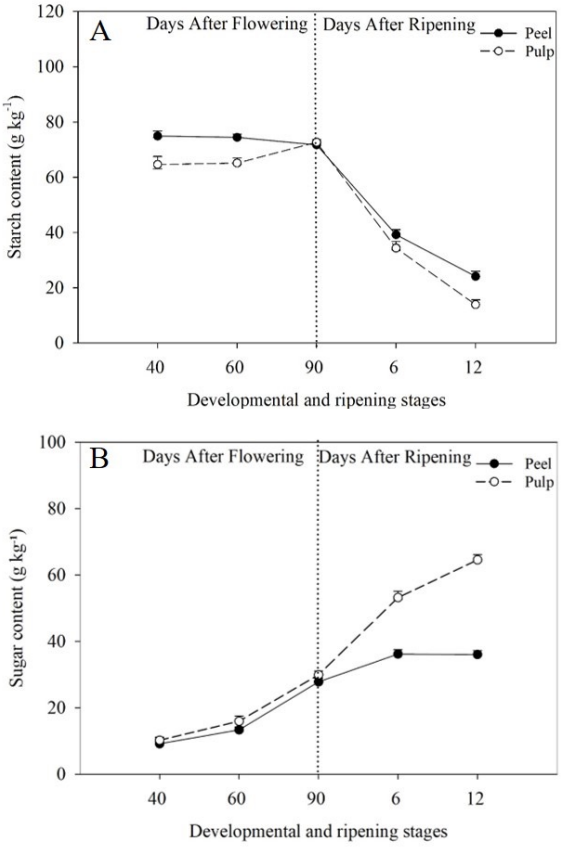


Fig. 3: Represents the biochemical changes in peel and pulp tissue of banana fruit during different developmental and ripening stages. Starch content**(A)** and total sugar**(B)** of banana peel and pulp tissues at different developmental and ripening stages, and expressed in g kg^{-1}.Each valuerepresents the mean of three biological replications of three fruits analyzed at each ripening stage and vertical error bars represent the standard deviation (±SD).

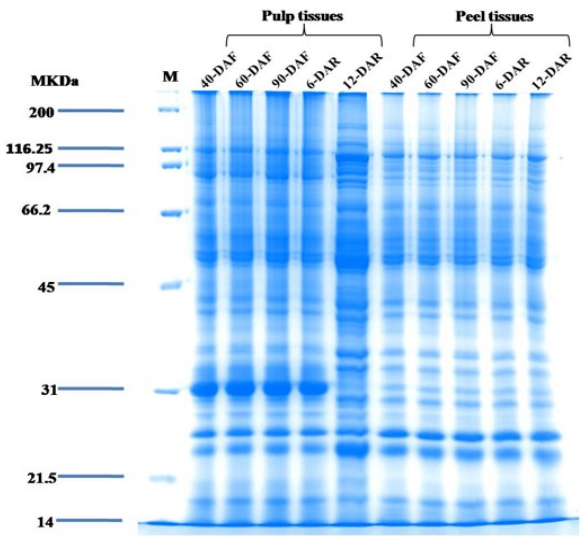


Fig. 4: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile showing separation of proteins from banana pulp and peel tissues of different developmental and ripening stages by using phenol-based extraction method. Known amount of protein (100µg) was loaded in each lane and resolved on 12% SDS-PAGE followed by Colloidal Coomassie blue staining. M: SDS-PAGE molecular weight standards (KDa), DAF: Days After Flowering, DAR: Days After Flowering.

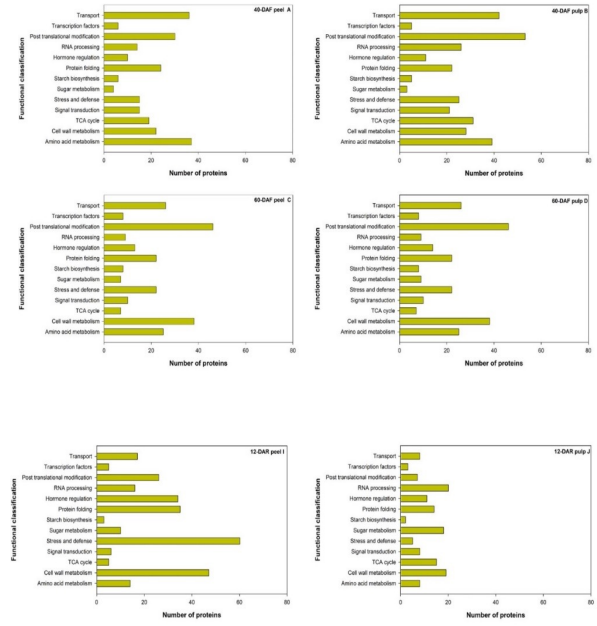


Fig. 5: Functional categories of the identified proteins from banana fruit tissues at different developmental and ripening stage represented as 40-DAF peel **(A)**, 40-DAF pulp **(B)**, 60-DAF peel **(C)**, 60-DAF pulp **(D)**, 90-DAF peel **(E)**, 90-DAF pulp **(F)**, 6-DAR peel **(G)**, 6-DAR pulp **(H)**, 12-DAR peel **(I)**, 12-DAR pulp **(J)**, through Orbitrap fusion mass spectrometry (mass spectrometer combines best of quadrupole, orbitrap and linear ion trap; tribrid) analysis.

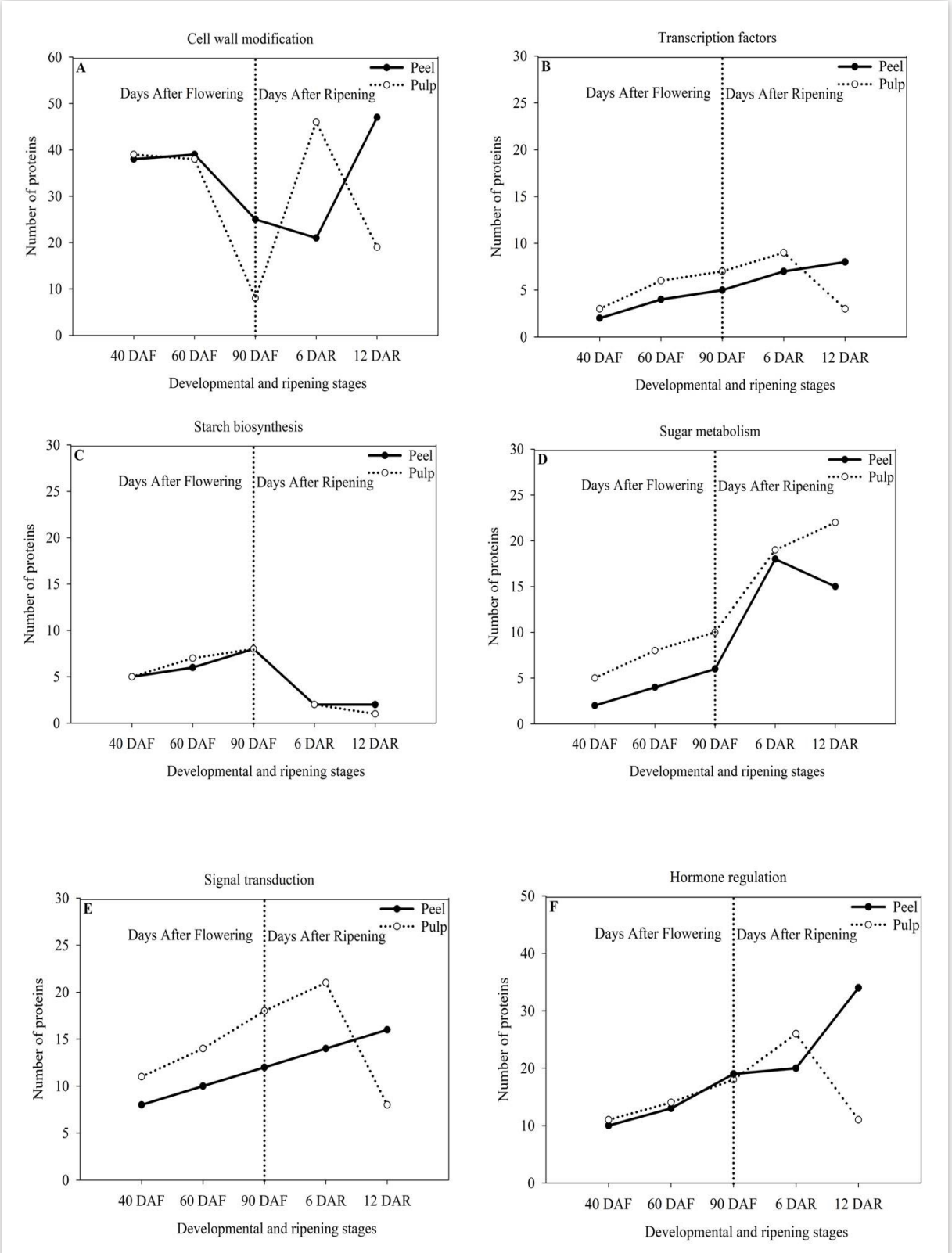


Fig. 6: Spatiotemporal expression of functionally categorized proteins those are identified from banana peel and pulp tissues at different developmental and ripening stages. Cell wall modification (A), transcription factors (B), starch biosynthesis (C), Sugar metabolism (D), signal transduction (E), hormone regulation (F).

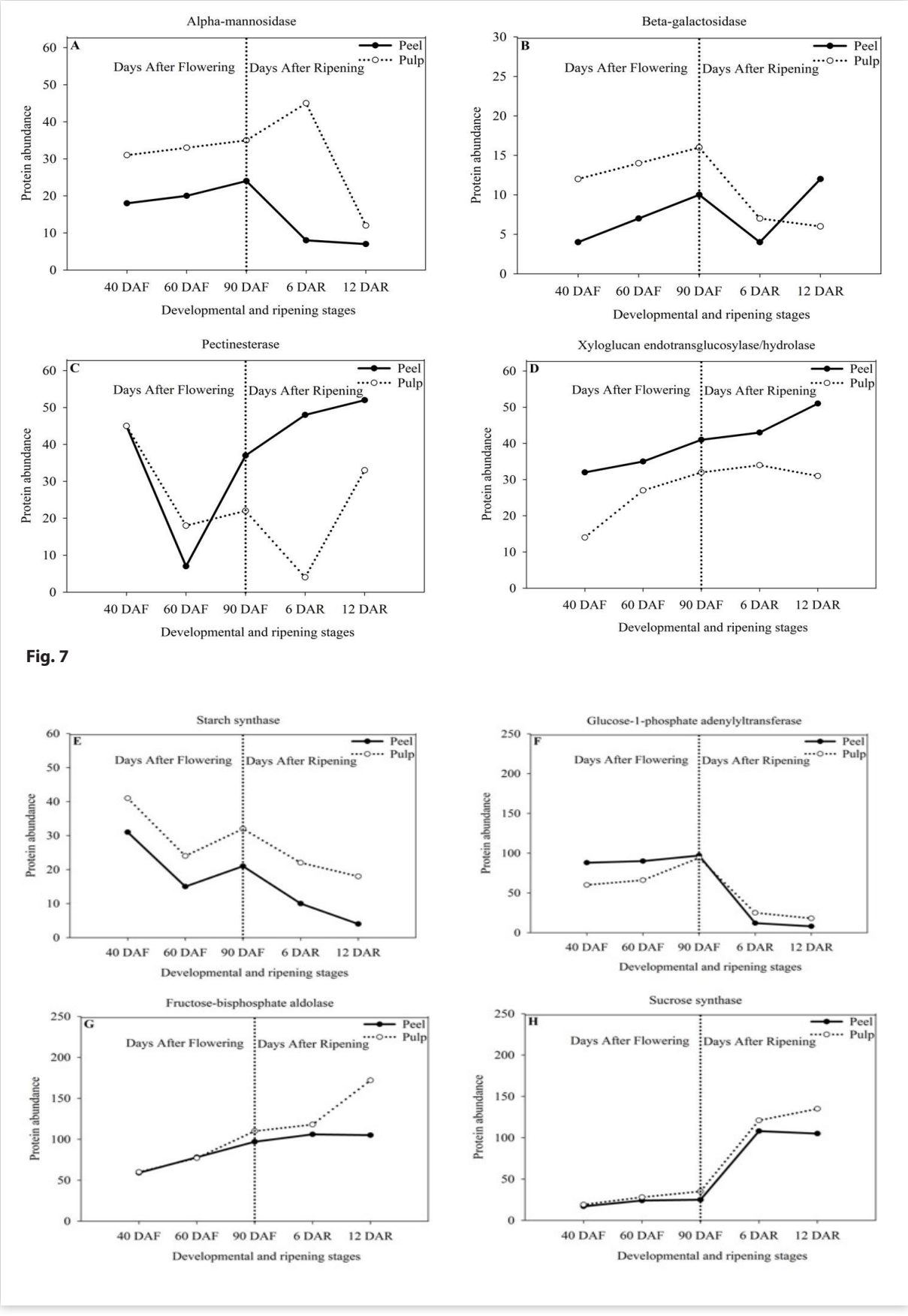

Alpha-mannosidase
A
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
10
20
30
40
50
60
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Beta-galactosidase
B
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
5
10
15
20
25
30
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Pectinesterase
C
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
10
20
30
40
50
60
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Xyloglucan endotransglucosylase/hydrolase
D
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
10
20
30
40
50
60
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Starch synthase
E
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
10
20
30
40
50
60
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Glucose-1-phosphate adenylyltransferase
F
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
50
100
150
200
250
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Fructose-bisphosphate aldolase
G
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
50
100
150
200
250
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages
Sucrose synthase
H
Peel
Pulp
Days After Flowering
Days After Ripening
Protein abundance
0
50
100
150
200
250
40 DAF
60 DAF
90 DAF
6 DAR
12 DAR
Developmental and ripening stages

Fig. 7

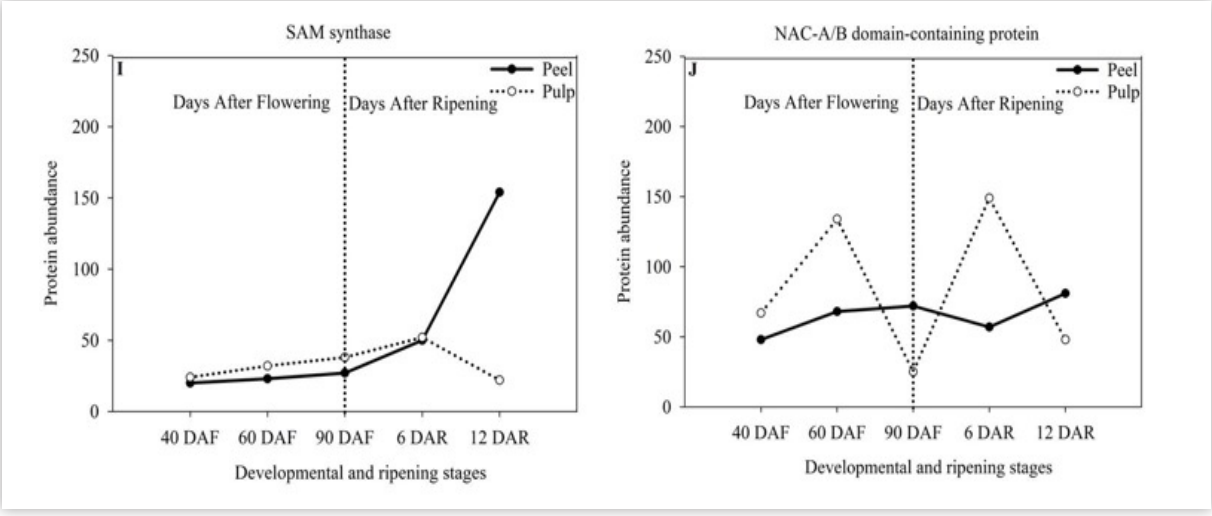


Fig. b

7A & B: Spatiotemporal expression analyses of individual proteins from banana peel and pulp tissues at different developmental and ripening stages. Proteins involved in different metabolic and regulatory pathways during developmental and ripening stages of banana were found to be differentially expressed. α-Mannosidase (α-man) (A), β-Galactosidase (β-gal) (B), Pectinesterases (PE) (C), Xyloglucan endotransglucosylase/hydrolase (XTH) (D), Starch synthase (SS) (E), Glucose-1-phosphate adenyltransferase (F), Fructose bis-phosphate aldolase (G), Sucrose synthase (H), SAM synthase (I), NAC-A/B domain-containing protein (J). Cell wall modified proteins: Pectinesterases, Alpha-mannosidases, Xyloglucan endotransglucosylase/hydrolaseand Beta-galactosidase. Starch metabolism proteins: Starch synthase and Glucose-1-phosphate adenyltransferase. Sugar metabolism proteins: Sucrose synthase, fructose bis-phosphate aldolase. Transcription factors: NAC-A/B domain containing proteins. Ethylene biosynthesis: SAM synthase.

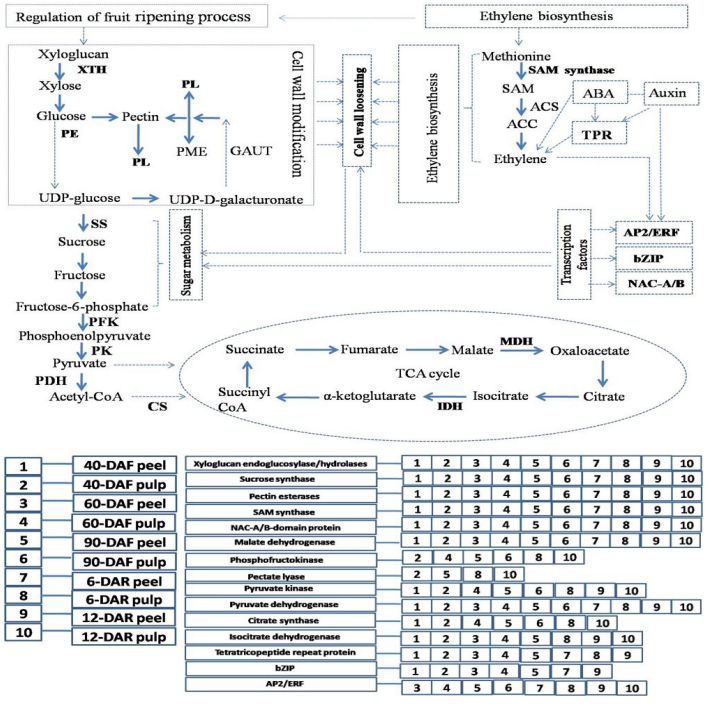


Fig. 8: Schematic overview of the proteins and their regulation in different pathways during banana fruit development and ripening process. Identified proteins were represented in bold letter which were involved in cell wall modification, sugar metabolism, TCA cycle, transcription factors, ethylene biosynthesis and hormone regulation. Cell wall modification proteins:Xyloglucan endotransglucosylase (XTH), pectate lyase (PL), pectin esterase (PE),sugar metabolism proteins:sucrose synthase, TCA cycle:phosphofructokinase (PFK), pyruvate kinase (PK), pyruvate dehydrogenase (PDH), citrate synthase (CS), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH), Ethylene biosynthesis: SAM synthase, Transcription factors: AP2/ERF, bZIP and NAC-A/B, Auxin and ABA signalling: TPR protein. Identified proteins were present in specific stage and tissue type.Different digits/codes were assigned for banana pulp and peel tissues of different developmental and ripening stages (1-10).

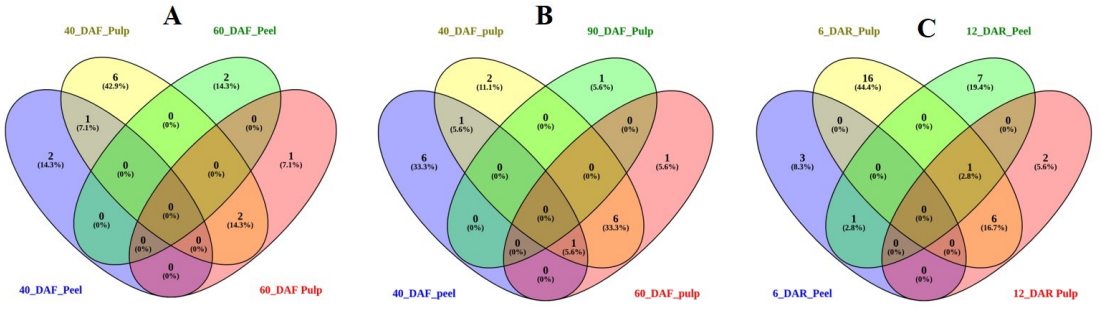


Fig. 9: Venn diagram exhibiting the commonality and uniqueness of identified proteins in different tissues and stages of banana fruit. The comparisons were made between 40 and 60-DAF peel and pulp tissue (A), 40 and 90-DAF peel and pulp tissue (B), 6 and 12-DAR peel and pulp tissue (C).Top 10 highly expressed proteinswere considered for the comparison.

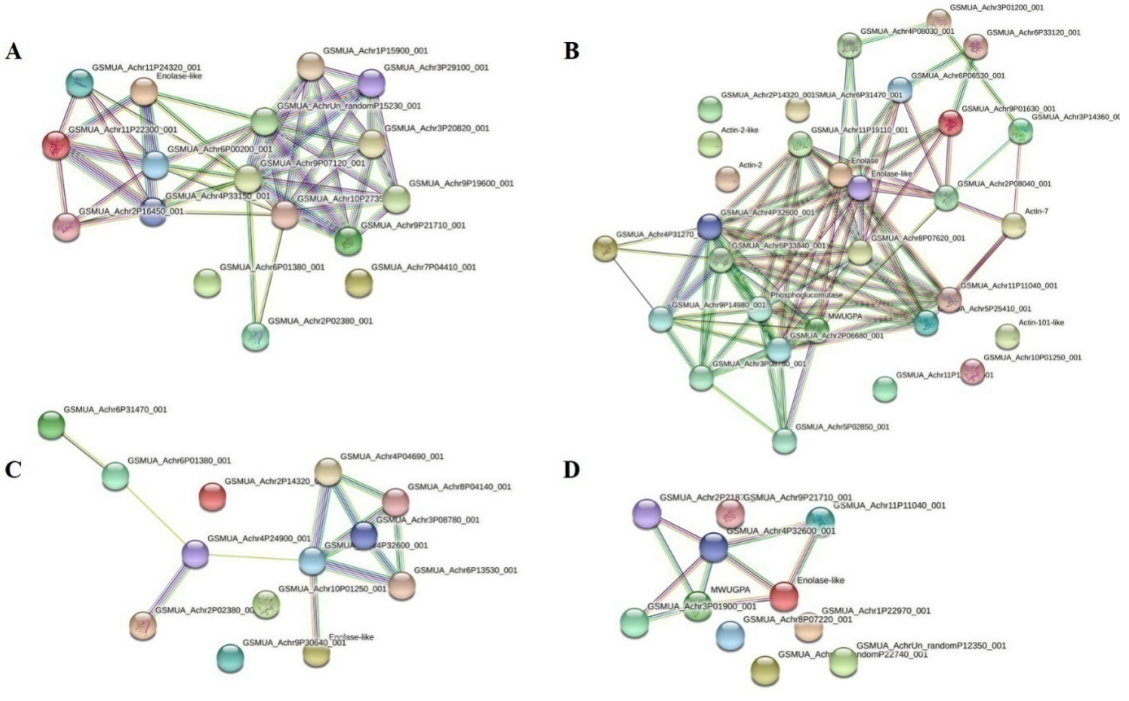


Fig. 10: The protein-protein interaction network simulated by STRING. Protein-protein interaction is presented for the identified proteins (top 20) in banana during different developmental and ripening stages. *Arabidopsis thaliana*network data base and confidence level of 0.4 were used for the analysis parameters. Different line colours represent the types of evidence used in predicting the associations. The comparisons were made between 6 and 12 DAR peel (A), 6 and 12 DAR pulp (B), 40, 60 and 90-DAF peel (C) and 40, 60 and 90-DAF pulp (D).

4.8 Developing efficient micro-propagation methods for some RET listed forest tree species of Odisha

Principal Investigator: Dr.Giridara Kumar Surabhi, Senior Scientist

(a). *P. santalinus L.* popularly known as red sanders, belonging to the family Fabaceae, is endemic to the southern parts of Eastern Ghats. IUCN has listed this plant as endangered. Compared to a very high flower production, the fruit set is very low. It is slow growing with prolonged juvenile period and late to reach seed-bearing age. Seed propagation in red sanders is posed with many constraints such as poor pod set, hard fruit coat, and less germinability coupled with poor viability. In addition, *P. santalinus* seeds are highly recalcitrant with a dormancy period of 1-year. Clonal multiplication through rooted cuttings is also an obstinate task in red sanders. When compared to seed germination experiments, there are almost no studies on *ex vitro* vegetative propagation in red sanders. Conventional vegetative propagation methods such as semi hard wood cuttings, cleft grafting, and air layering were executed with varying degrees of success and has limitations in producing enough numbers of planting material for forestry programs. Culturing mature tree explants needs to be preferred over seedling explants, because it is often not possible to determine whether these embryos or seedlings have the genetic potential to develop the desired qualities and because of their heterozygous nature.

(b). *L. glutinosa*(Lour) C.B is an evergreen tree of medium size which grows to a height of about 20 to 30 feet, belongs to the family Lauraceae. *L. glutinosa* is a medium sized evergreen tree, grows up to 25m high and 1.5m in girth and a clean bole of around 5-6m. it is found throughout India ascending up to an altitude of 1400m in the Himalayas. Its conservation status had been variously assessed a critically endangered, and endangered. It is distributed in Eastern Ghats, including parts of Odisha. The leaves and mucilage from the bark of plant is utilized in gum for poultices. The seeds contain an aromatic oil, which is used to make candles and soap. The roots yield fibers used in rope manufacture and for paper pulp. The fruit is a small red, purple or black drupe containing a single seed, dispersed mostly by birds. The fruits have a sweet creamy edible pulp that can be taken as food. The conventional propagation is hampered due to low seed viability and no rooting of vegetative cuttings. Thus, there is need for alternative in vitro propagation method to develop a procedure for its micropropagation for multiplication, improvement and conservation of the species.

(c). *L. comberi* Haines is an endangered forest tree species. *L. comberi* is a moderate-sized evergreen tree belonging to the family Euphorbiaceae and has very narrow distribution range in Eastern India and Thailand.*L. comberi* occurs in deep ravines and hill slopes closer to streams and can be easily identified by its characteristic whitish-grey, deeply fissured trunks.

(d). *P. simiarum*(Hook. F. &Thomson) is an endangered forest tree species belonging to the familyAnnonaceae. The plant *P. simiarum* is known to exhibit antimicrobial and cytotoxic activities. Literature survey indicated that, no studies had been initiated on micropropagation of this endangered forest tree species. Micro-propagation has proven to be the method of choice for rapid multiplication of selected RET listed forest tree species, where seed and vegetative propagation is a problem. To this end, present study would be highly useful in developing efficient *in vitro* propagation methods for important RET listed forest tree species of Odisha, to support re-introduction and restoration programs.

(i). *Pterocarpus santalinus*:

In vitro seed germination and growth of*P. santalinus*:

After completion of the surface sterilization procedure the seeds wereplaced in the media (1 seed per culture bottle), each culture bottle containing 60 ml of MS media supplemented with or without 1.0 - 1.5 mg/L BAP (i.e., 6-benzylaminopurine- a plant growth regulator), 30 mg/L sucrose, and 7 g/L agar).

Shoot induction and growthof P. santalinus:

Nodal explantsi.e., 1.5-2.0 cm (collected from *in vitro* germinated seedlings) were inoculated in the culture media. The shoot induction media prepared in the following combinations i.e.,MS media supplemented with different combinations of NAA (0.5 and 1.0 mg/L) and BAP(1.0 and1.5 mg/L)with or without citric acid (22.689 mg/L), activated charcoal (100-150 mg/L) for obtaining shoot induction and growth.

Shoot elongation and growthof *P. santalinus:*

The optimum concentration for multiple shoot induction and subsequent growth, various concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) were used in Murashige and Skoog (MS) medium (MurashigeandSkoog 1962) with sucrose (3%).The microshoots arise from the nodal shoot segments (in vitro grown shoots) werefurther transferred (subculture) to new culture medium (MS medium)having same combination or different combination of plant growth regulators (1.0 and 1.5 mg/L benzylaminopurine (BAP); 1.0 mg/L naphthaleneacetic acid (NAA) along with additives (i.e., 22.689 mg/L citric acid and activated charcoal 100 mg/L) for shoot multiplication.

Root induction:

Shoots of 3.0-5.0 cm in length was used for *in vitro* root induction. Micro shoots were subsequently transferred to MS (half strength or full strength) medium supplemented with various concentrations of auxins: IBA (indole-3-butyric acid) and IAA (indole-3-acetic acid).

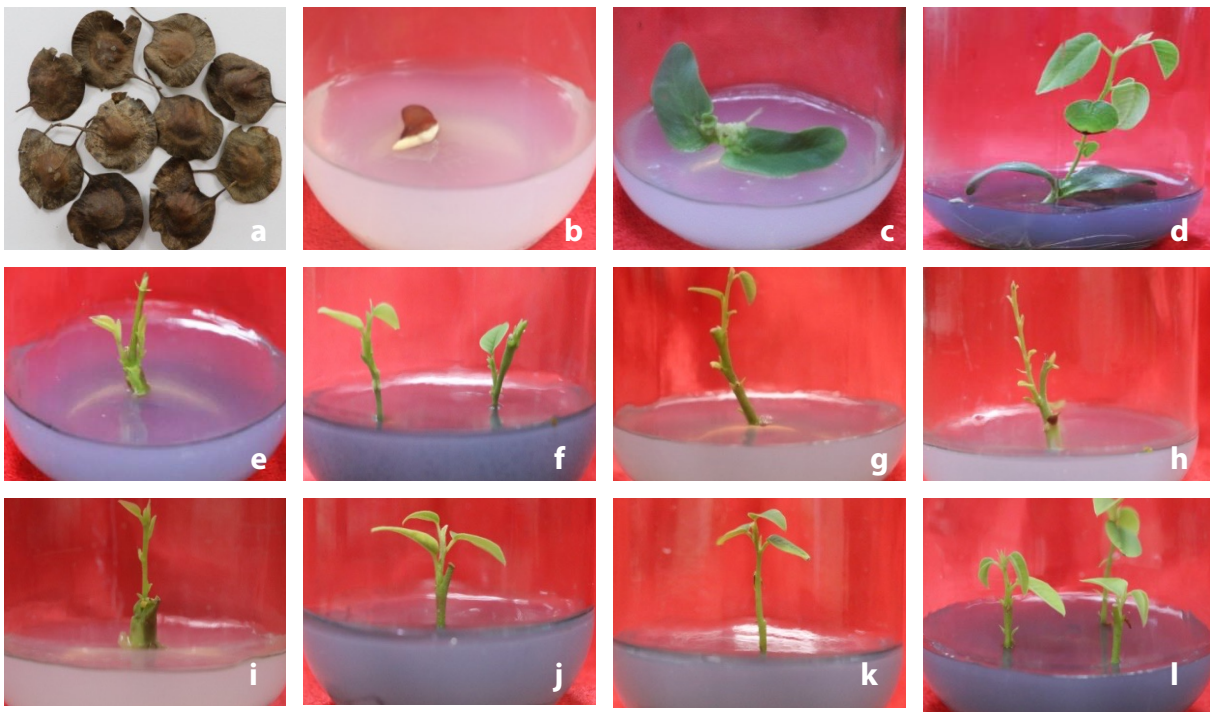


Fig. 1: (A). Pods of *P. santalinus,*(B). Seed germination of *P. santalinus* inoculated in MS media supplemented with 1.0mg/L BAP, (C) Seedling formed with cotyledons, inter node, axillary node, hypocotyl, (D). Seedling established with 5-6 nodes after 30-days of inoculation on MS media supplemented with 2.0mg/L BAP+ NAA (0.5 mg/L)+ ascorbic acid (50mg/L) + citric acid (22.689mg/L) + activated charcoal (100mg/L), (E&F). Shoot induction from nodal explants (germinated seedlings) observed after 17-days of inoculation on MS media supplemented with 2.0mg/L BAP, ascorbic acid (50mg/L) + citric acid (22.689mg/L) + activated charcoal (100mg/L), (G&H). shoot induction were observed after 30-days of inoculation on MS media supplemented with 1.0mg/L BAP + citric acid (22.689mg/L+NAA(0.5mg/L) +activated charcoal (100mg/L), (I-L). shoot growth was observed after 45 days of inoculation on MS media supplemented with 1.0mg/L BAP + citric acid (22.689mg/L)+activated charcoal (100mg/L).

Shoot elongation and growth of *P.santalinu* through seedlings:

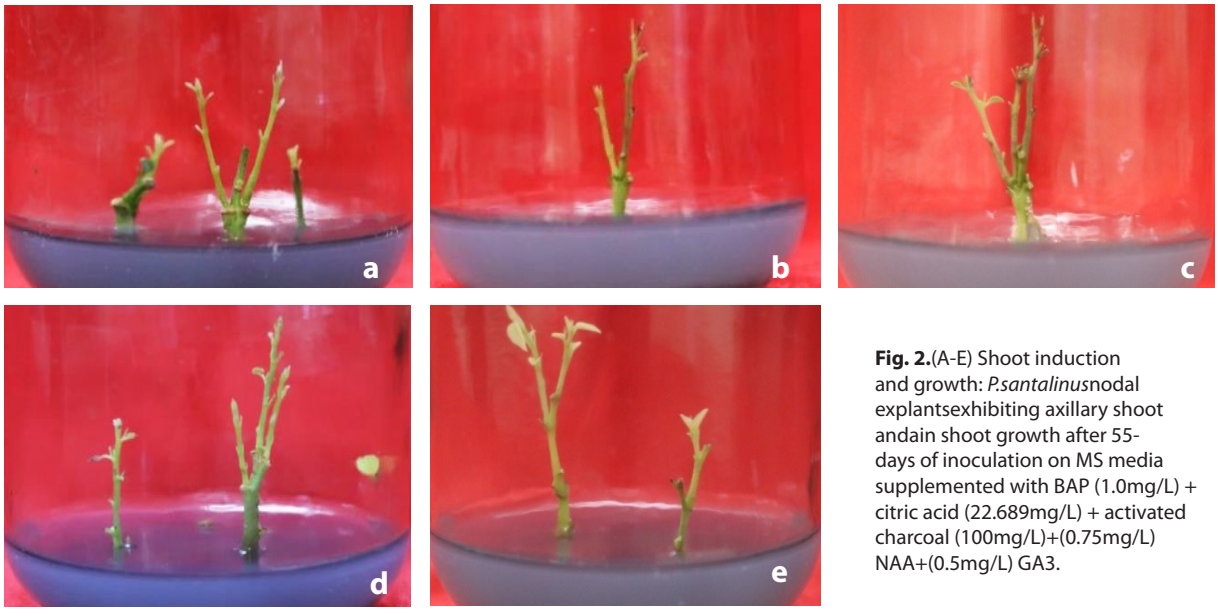


Fig. 2.(A-E) Shoot induction and growth: *P.santalinus*nodal explantsexhibiting axillary shoot andain shoot growth after 55-days of inoculation on MS media supplemented with BAP (1.0mg/L) + citric acid (22.689mg/L) + activated charcoal (100mg/L)+(0.75mg/L) NAA+(0.5mg/L) GA3.

Shoot initiation and growth of L.comberi

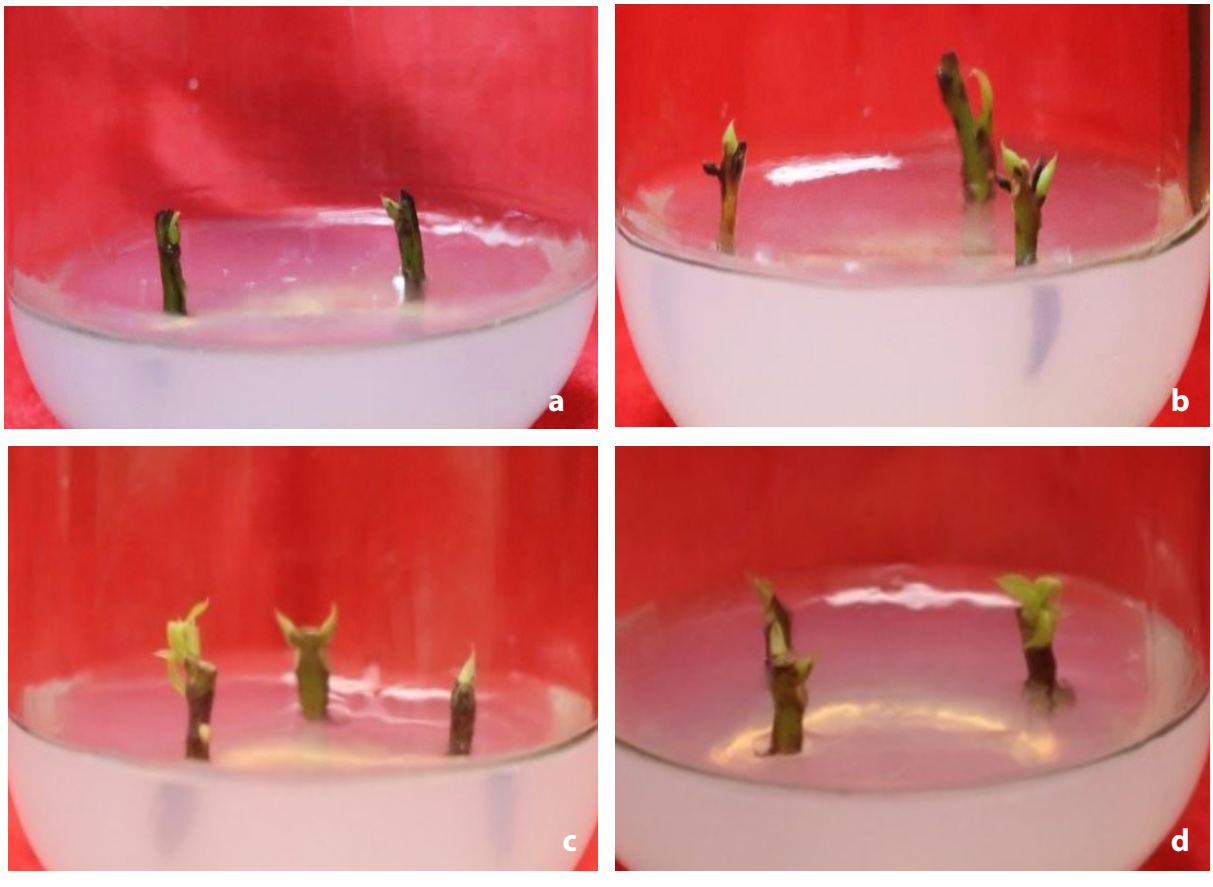


Fig. 4.: (A) and (B) Explants of *L.comberi*showed shoot induction after 10-days of inoculation on MS media supplemented with 1.0 mg/L BAP.(C) and (D) *L.comberi*showed shoot induction after 20-days of inoculation on MS media supplemented with 1.0 mg/L BAP.

4.9 Ameliorative effect of *Aporosa octandra* against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental rats.

Principal Investigator: Dr. Atish Kumar Sahoo, Senior Scientist

Aporosa octandra is a native species of India, Assam, Bangladesh, Cambodia, China South-Central, East Himalaya. It is commonly known as "Indo-China Alder", "Chhawntual", "Massania", "Kharalla", "Kasua", that belongs to the family Euphorbiaceae, sub-family Phyllanthaceae which is shrub to tree, up to 15 m high and comprises of 50 species. Different parts of the plant are used for the medicinal purpose by the tribal peoples of south-eastern part of India. However, the biological properties of *A. octandra* have not been studied well. The plants of this genus are reported to possess various medicinal properties. Traditionally, a decoction of the *A. octandra* bark (5-15 mL, 3-5 times daily) is used orally for treating oxidative stress, inflammation, peptic ulcer, and hypotension. Although this plant is used for many years as a traditional medicinal plant yet its phytochemical investigation is not reported to date and also this plant has never been screened for any oxidative stress studies. Therefore, the present study considers the bioassay of the extracts of its leaf to explore Ameliorative effect of *Aporosa octandra* against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental animal model. We have also considered the phytochemical investigation of the extracts

Sample collection and extraction

The leaves of *A. octandra* was collected from Barbera Forest division under Khordha district of Odisha, India. The leaves were cleaned and shed dried under room temperature. The sample was prepared for extraction by grinded coarse powder form (500 g) and divided in to three, one part was taken for the successive extraction based on the polarity index of the solvent (Hexane- chloroform-ethyl acetate-methanol) in a Soxhlet apparatus. The extraction was carried out until the solvent in the extractor became colorless. Another part was taken for hydroalcohol (70%) hot maceration process. The powered leaf material was taken in 1:4 ratio with hydroalcohol and boiled at 60-80 ^{o}C for 72h. In each 3h interval the filtrate was collected and filtered. The remaining solvent in the extract was removed at reduced pressure and temperature using a rotary evaporator. The semi solid sample from all the three extractions were collected and kept for further preliminary screening and other experiment purpose. The extraction was completed and the yield derived was given in Table 1.

Table 1. Percentage of yield of different extracts of *A octandra*

Plant Material	Extract	% of yield(gm)
***Aporosa octandra* (leaf)**	Hexane (AOHE)	0.81
	Chloroform (AOCL)	0.61
	Ethyl acetate (AOEA)	0.4
	Methanol (AOME)	2.91
	Hydroalcohol (AOHA)	15.13

Preliminary screening of *A. octandra*

Phytochemical estimation revealed the presence and absence of different phytoconstituents. Different type of preliminary screening tests was carried out to detect the presence and absence of different phytoconstituents e.g., Phenols, flavonoids, steroids, alkaloids, glycosides, terpenoids, anthraquinones and tannins.

Table 2. Preliminary phytochemical screening of various extracts of *A. octandra*

Phytochemicals	Extracted fractions of *A. octandra*				
	AOHE	AOCL	AOEA	AOME	AOHA
Phenols	-	-	+	+++	++
Flavonoids	-	-	+	++	+
Steroids	-	-	++	-	-
Alkaloids	-	-	+	++	+
Glycosides	++	++	-	++	+
Terpenoids	-	-	-	++	+
Anthraquinones	-	-	-	+	+
Tannins	-	+	-	+	+

Estimation of secondary metabolites *and estimation*

Secondary metabolites such as total phenol content (TPC), total flavonoids content (TFC) and total alkaloid content (TAC) estimation of various solvent extracted fraction of *A. octandra* were conducted and the results were mentioned in Table 3. It was found that the hydroalcohol extract of *A. octandra* has highest quantity of secondary metabolites (TPC; 413.67 mg GAE/g extract, TFC; 927.52 mg QE/g extract, TAC; 1070.89 mg AT/g extract) as compared to the other extracted fraction.

Table 3. Secondary metabolites of *A. octandra*

Extracts	TPC (mg GAE/g)	TFC (mg QE/ g)	TAC (mg AT/g)
Hexane (AOHE)	1.94	1.89	2.11
Chloroform (AOCL)	2.90	1.23	2.02
Ethyl acetate (AOEA)	275.89	752.45	102.78
Methanol (AOME)	413.67	677.54	1380.98
Hydrocohol (AOHA)	332.23	927.52	1070.89

Establishment radical scavenging mechanism to counter cellular oxidants by performing DPPH, ORAC, and CAP-e assays DPPH free radical scavenging assay

The ability of *A. octandra* to scavenge DPPH free radical was studied (Kumara and Kakkar, 2008). The antioxidant activities of various fractions were determined on the basis of their scavenging abilities with the increase in concentration by forming stable DPPH. About 1 mL of DPPH solution (5.9 mg in 100 mL methanol) was added to 1 mL of different concentrations of various plant extract (10- 100 µg/mL). After the incubation period of 15-30 min, the absorbance at 517 nm was determined in microplate reader (Synergy H1MF, BioTek, USA). Ascorbic acid was used as a reference drug and % of inhibition was calculated by the following equation. IC_{50} is a parameter representing the extract concentration at which it can inhibit 50% of DPPH radicals. IC_{50} value

for each extract was obtained by constructing a graph with the sample concentration on the Y-axis and % of inhibition on the X-axis. The results of our current findings in DPPH assay found that the AOHA extract (IC_{50}; 36.4694± 1.09 µg/mL) demonstrated more inhibitory potential of free radicals than other extracts (Table 4; Fig.1)

Table 4. DPPH free radical inhibition effects of *A. octandra*

Extracts/ Reference drug	IC_{50} (µg/mL)
Ascorbic acid (reference drug)	21.67 ± 1.04
AOHE	197.8 ± 1.09
AOCL	187.30 ± 0.09
AOEA	45.8 ± 0.12
AOME	46.17 ± 0.19
AOHA	36.494± 1.09

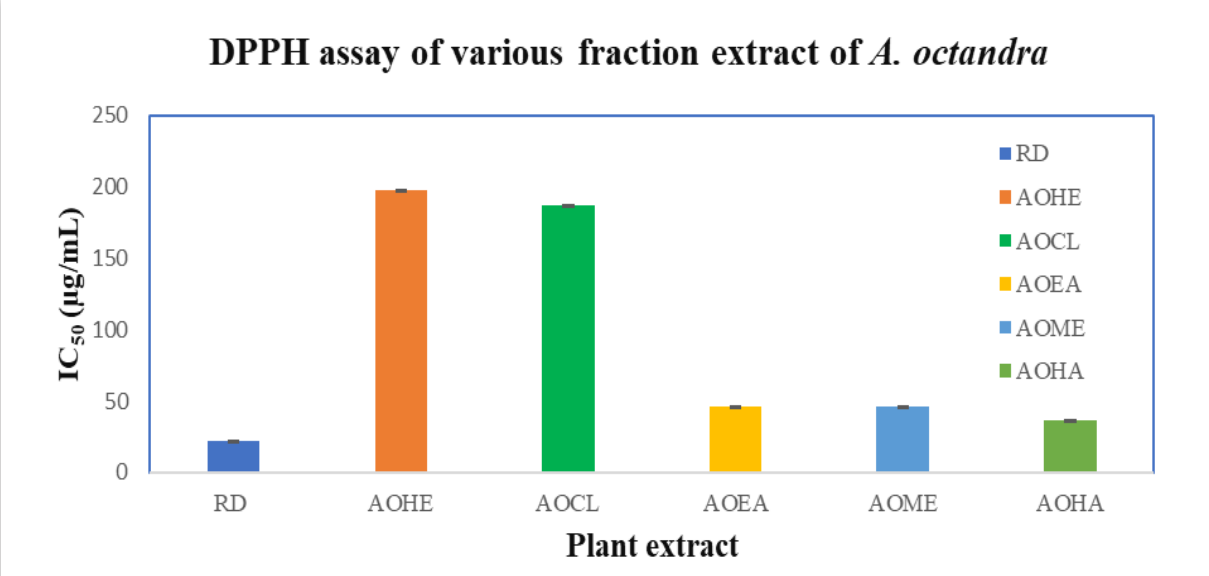


Fig.1. DPPH assay of various fractions of *A. octandra*

Total Antioxidant Capacity of *A. octandra* by ORAC assay

ORAC assay depends on assessing the effect of presumed antioxidants by measuring the fluorescence quenching and based on *in situ* production of peroxyl free radicals generated via AAPH. Peroxyl radical was able to react with oxidizable substrate and increase the rate of fluorescence decay. Antioxidant potential of extracts of *A. octandra* was expressed in form of net AUC. Net AUC for AOHA and reference drug Trolox was calculated to be 14.13, 11.15 and 20.87 respectively (Fig.2).

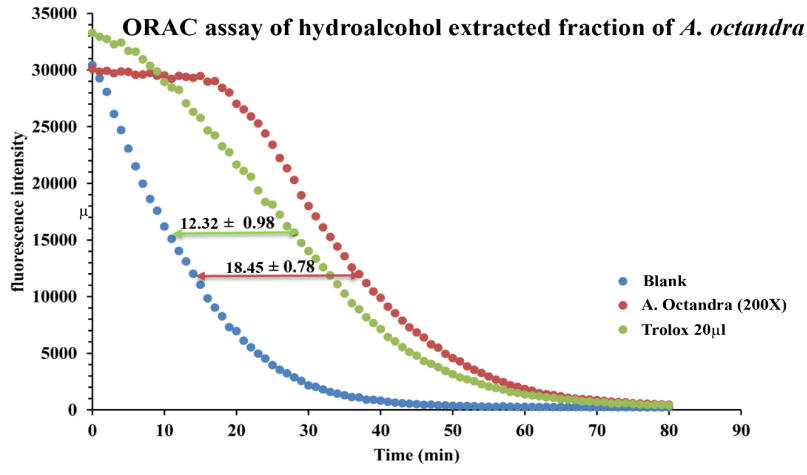


Fig 2. ORAC assay of AOHA of *A. octandra.*

Ex vivo Cellular antioxidant protection (CAP-e) test of of *A. octandra*

The study of cellular antioxidant protection (CAP-e) of AOHA of *A. octandra* was measured by using erythrocytes obtained from Wistar rats. % of Inhibition of oxidative damage by AOHA was calculated by comparing the cells treated with AAPH (oxidizing agent). AOHA demonstrated cellular antioxidant defence with IC_{50} 40.97 µg/mL, whereas the IC_{50} of standard drug gallic acid was 24.82 µg/mL (Table 5).

Table 5. *Ex vivo* CAP-e assay of hydroalcohol extracted fraction of *A. octandra*

Sl. no.	Sample	IC_{50} values (µg/mL)
1	AOHA of *A. octandra*	40.97 ± 0.68
2	RD (gallic acid)	24.82 ± 1.54

Acute toxicity study lethal dose (LD_{50}) and effective dose (ED_{50}) of *A. octandra*:

No mortality, or any difference in sign and symptoms was found in the AOHA treated group (I, II), while the ratio of death/treatment in group III, IV and V was 1/6, 1/6 and 3/6, respectively with symptoms of salivation, higher degree of irritation, restlessness, and increased urination (Table 6). As 50% rats were died at 4000 mg/kg b.w. thus, LD_{50} was estimated to be 4000 mg/kg b.w. and effective doses of AOHA were recorded as 300 and 400 mg/kg b.w. in rats (Table 6).

Table 6. Acute toxicity study of AOHA of *A. octandra*

Groups	Fraction	Dose (mg/kg)	D/T	Symptoms
I	AOHA	500	0/6	NA
II		1000	0/6	NA
III		2000	1/6	Defecation
IV		3000	1/6	Excess urination and irritation
V		4000	3/6	Death

4.10 The protective diabetic neuropathy effect of *Buchanaia lanzan* Spreng. in streptozotocin induced type 2 diabetic rats.

Principal Investigator: Dr. Atish Kumar Sahoo, Senior Scientist

The project was undertaken for exploring the therapeutic potential by evaluating the diabetic neuropathy effect of *Buchanania lanzan* Spreng (fruits) in Swiss albino rats. *Buchanania lanzan* Spreng. (Chironji/ Chara koli) (Anacardiaceae), 'Almondette tree' in English is distributed in tropical deciduous forests of North, Western and Central India mostly with a monsoonal climate. The tree grows most commonly on yellow sandy-loam soil. Fruit is a yellowish-red drupe, one seeded, ripens from April to May. In Ayurvedic System of Medicine (ASM), various part this plant used as astringent, glandular swelling, cardiotonic, and cancer. The phytoconstituent e.g., tannins, quercetin, gallic acid and glucoside were reported in bark of this plant. However, the traditional claim of *B. lanzan* as brain tonic to improve memory has not been scientifically validated yet. So, the project proposed to establish the protective diabetic neuropathy effect of *B. lanzan* in streptozotocin induced diabetic rats.

Nowadays, the effect of diabetes on the central nervous system (CNS) is gaining importance. The brain cholinergic signalling is implicated in the regulation of cytokines release, hepatic glucose/glycogen production via efferent vagus nerve cholinergic output and acetylcholine (ACh) facilitates the release of insulin in a glucose-dependent manner. It is likely that these vagus nerve-mediated cholinergic mechanisms, involved in glucose homeostasis become suppressed, or dysfunctional in obesity and insulin resistance, as indicated by the autonomic imbalance and lower vagal tone in obese individuals. Moreover, AChE activity will be proven to increase in diabetic models, which indicates alteration in the cholinergic neurotransmission with the consequent cognitive impairments observed in the diabetic state. Hence, it is now plausible to target AChE to increase the cholinergic pathway activity, could be beneficial in the management of T2DM.

Sample collection and extraction

The fruits of the plant *Buchanania lanzan* Spreng (fruits) was collected from Keonjhar and Sundergarh district of Odisha, India. The fruits of *B. lanzan* were washed, cleaned and shed dried under room temperature. The sample was prepared for extraction by grinded coarse powder form (1500 g) and divided in to three, one part was taken for the successive extraction based on the polarity index of the solvent (Hexane-chloroform-ethyl acetate-methanol) in a Soxhlet apparatus, and the extraction was carried out until the solvent in the extractor became colorless. Another part was taken for hydroalcohol (70%) hot maceration process, the powered fruit material was taken in 1:4 ratio with hydroalcohol and boiled at 60-80 ^{o}C for 72h. In each 3h interval the filtrate was collected and filtered. The remaining solvent in the extract was removed at reduced pressure and temperature using a rotary evaporator. The last part was taken for water extract with hot maceration extraction. The semi solid sample from all the extractions were collected and kept for further preliminary screening and other experiment purpose. The extraction was completed and the yield derived was given below.

Table 1. Percentage of yield of different extract of *B. lanzan*

Plant Material	Extract	% Of yield(gm)
***Buchanania lanzan* (Fruit)**	Hexane (BLHE)	0.5
	Chloroform (BLCL)	8
	Ethyl acetate (BLEA)	8
	Methanol (BLME)	7
	Water (BLWA)	10
	Hydroalcohol (BLHA)	13.46

Preliminary screening of *B. lanzan*

Screening of solvent extracts of *B. lanzan* clearly revealed that the maximum classes of phytoconstituents are present in hydroalcohol (BLHA) extract as compared to other extracts. Hence, BLHA could have been the therapeutic significance extract for further consideration. Furthermore, quantitative analyses of these phytocompounds were carried out.

Table 2. Preliminary phytochemical screening of various extracts of *B. lanzan*

Phytochemicals	Extract Fractions of *B. lanzan*				
	BLHA	BLWA	BLME	BLEA	BLCL
Phenols	+++	++	+	+	-
Flavonoids	+++	++	+	+	-
Steroids	++	++	++	-	+
Alkaloids	+++	++	+	+	-
Glycosides	++	-	+	-	+
Terpenoids	-	-	-	-	-
Anthraquinones	++	-	-	-	-
Tannins	++	+	++	++	-

Estimation of secondary metabolites

Secondary metabolites such as total phenol (TPC), total flavonoids (TFC) and total alkaloid content (TAC) estimation of various solvent extracted fraction of *B. lanzan* were conducted and the results were mentioned in Table. 3. It was found that the hydroalcohol fraction extract of *B. lanzan* had highest quantity secondary metabolites (TPC; 546.99 mg GAE/g extract, TFC; 670.99 mg QE/g extract, TAC; 908.05 mg AT/g extract) as compared than extracts.

Table 3. Secondary metabolites of *B. lanzan*

Extracts	TPC (mg/g eqv. Of Gallic Acid)	TFC (mg/g eqv. Of Quercetin)	TAC (mg/g eqv. Of Atropine)
Ethyl acetate (BLEA)	217.97	590.23	259.45
Methanol (BLME)	219.61	310.11	536.6
Hydroalcohol (BLHA)	546.99	670.99	908.05
Water (BLWA)	324.78	345.99	560.97

Establish free radical scavenging activities of *B. lanzan*

DPPH free radical scavenging assay

The ability of *B. lanzan* to scavenge DPPH free radicals was studied (Kumara and Kakkar, 2008). The antioxidant activity of various fractions was determined on the basis of their scavenging abilities with the increase in concentration by forming stable DPPH. IC_{50} value was obtained by constructing a graph with the sample concentration on the Y-axis and % of inhibition on the X-axis. It was found that BLHA (IC_{50}; 57.46 ± 1.09 µg/mL) has more inhibitory potential of scavenging free radicals than other extract (Fig.1).

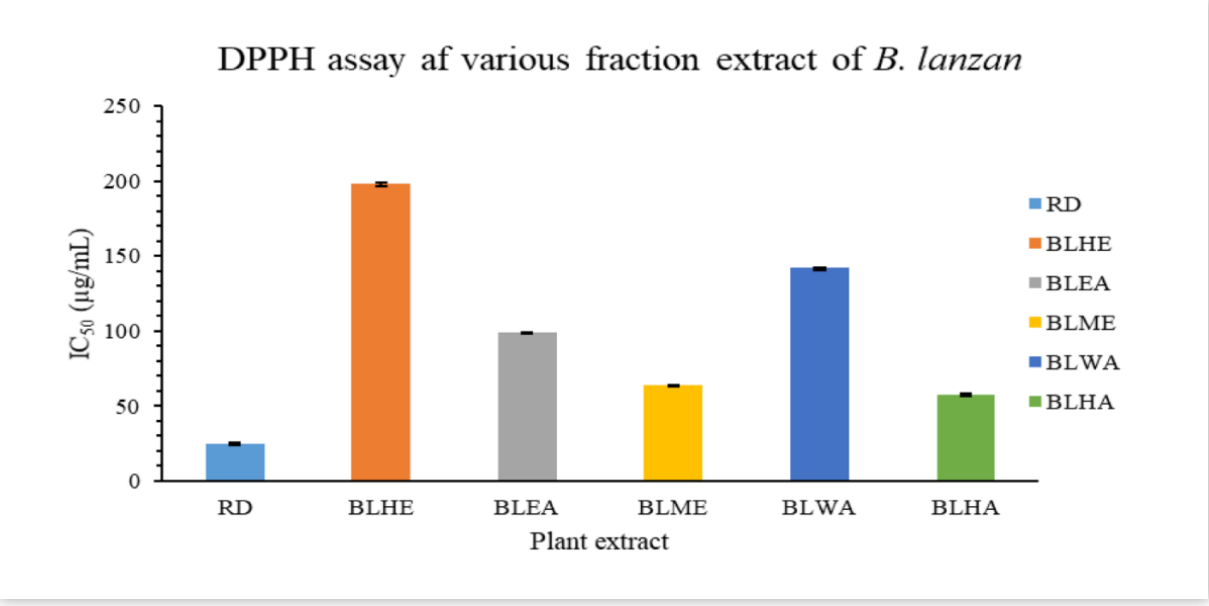


Fig 1. DPPH antioxidant assay of *B. lanzan*.

In vitro* antidiabetic inhibition assay α-amylase activity of *B. lanzan

The control of postprandial plasma glucose level is important in the treatment of diabetes mellitus and it is associated various complications. Inhibition of enzymes involved in the metabolism of carbohydrates such as α-amylase and α-glucosidase, is an important therapeutic approach for reducing postprandial hyperglycemia. α-amylase found in the saliva and pancreatic juice, catalyzes the hydrolysis of polysaccharides (such as starch) to disaccharides (like maltose and sucrose) (Swain et al., 2020). From the result of α-amylase inhibition assay, it was found that BLHA showed better enzymatic activity with IC_{50} 46.62 ± 0.89 µg/mL as compared to other extract.

Table 5. α-amylase activity of different extract of *B. lanzan*.

Extracts/ reference drug	IC_{50} (µg/mL)
Acarbose (reference drug)	24.48 ± 0.87
BLCL	111 ± 1.12
BLEA	65.76 ± 0.45
BLME	85.66 ± 1.09
BLWA	84.52 ± 1.07
BLHA	46.62 ± 0.89

α-glucosidase inhibitory activity of *B. lanzan*

α-glucosidases are located in the mucosal brush border of the small intestine and catalyze the conversion of disaccharides to monosaccharides. In this study, hydroalcohol (BLHA) extract of *B. lanzan* exhibited the strongest inhibition (IC_{50}; 67.46 ± 0.45 µg/mL) of α-glucosidase than other extract.

The current findings suggested that *B. lanzan* could be an efficient antioxidant agent which were responsible for potential antidiabetic activities (Mollica et al.,2018).

Table 6. α-glucosidase inhibitory activity of *B. lanzan*.

Extracts	IC_{50} (µg/mL)
Acarbose (reference drug)	30.01 ± 0.12
BLCL	120.83 ± 0.98
BLEA	115 ± 1.12
BLME	86.54 ± 1.05
BLWA	96.12 ± 1.23
BLHA	67.46 ± 0.45

Acute toxicity study

Different concentration of extract was given to rats by oral gavage to overnight fasted animals. All animals were observed individually for mortality and general changes. Toxicity sign and symptoms such as alertness, restlessness, irritability, fearfulness, touch response, gait, defecation, and urination were observed for 72 h and LD_{50} value of BLHA was calculated. Animal experiment was done with 40 Wistar albino rats of either sex was taken and divided into two dietary groups.

Table 7. Acute toxicity study.

Groups	Fraction	Dose (mg/kg)	D/T	Symptoms
I	**BLHA**	500	0/6	NA
II		1000	0/6	NA
III		2000	0/6	NA
IV		3000	1/6	Excess urination and irritation
V		5000	3/6	Death

***In vivo* experimentation**

The animals were randomly allocated into 5 groups (n = 6) as follows:

- Group I served as normal control were given received 0.9% normal saline (1 mL/kg).
- Group II served as toxic control were given received 0.9% normal saline.
- Groups III served as positive control will be received pioglitazone-HCl (Oral, 4 mg/kg, p.o.).
- Group-IV and V served as lower and higher dose of bioactive fraction/ molecule of *B. lanzan* (BLHA) (Oral, Low and High doses mg/kg, p.o.) will be treated diabetic rats, respectively.

Oral glucose tolerance test (OGTT)

OGTT was performed on the 15th day of the experiment. We observed no significant change in SG level (81.93 ± 1.78 mg/dL) and AUC (7986) in normal group rats at 2 h post oral glucose administration and this was due to the enhanced release of insulin (Fig.1A-B).

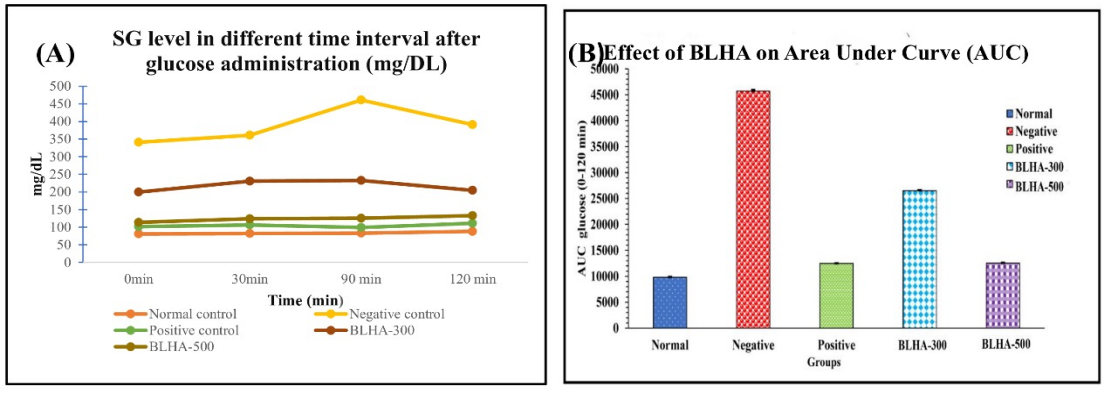


Fig.1. Oral glucose tolerance test.(A) SG level in different time interval after glucose administration (mg/DL). (B) Effect of BLHA on Area Under Curve (AUC).

Estimation of serum oxidative marker

SGOT and SGPT are associated with the occurrence of T2DM, on completion of 28 days study period, the level of SGOT (Fig. 2A), SGPT (Fig. 2B)) elevated in negative control as compared to normal. By comparing the results from this study, BLHA-500 mg/kg showed

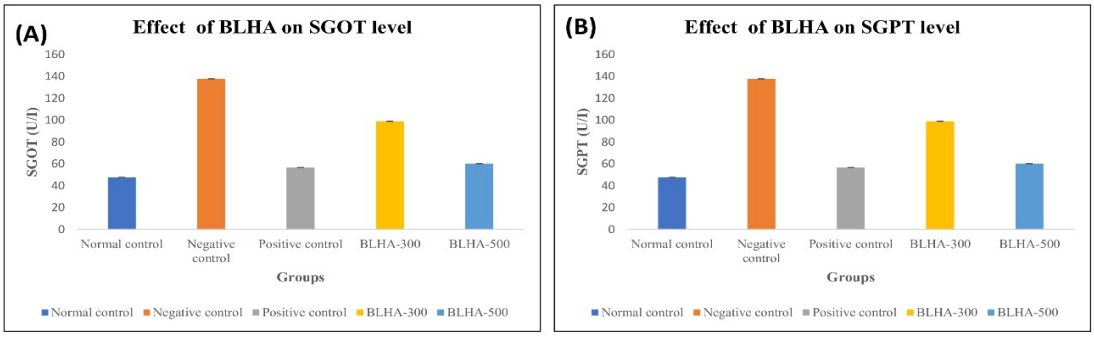


Fig. 2. Demonstrating (A) Effect of BLHA on SGOT level (B) Effect of BLHA on SGPT level

significant ($p < 0.001$) reduction in SGOT (61.33 ± 1.52 U/L), SGPT (25.30 ± 0.15 U/L).

Estimate the lipid profile of serum sample:

TG level significantly ($p < 0.001$) elevated 315.33 ± 2.52 mg/dL) in STZ induced diabetic rats as compared to the normal (120.35 ± 1.52 mg/dL). But, pre-treatment of BLHA-500 mg/kg significantly ($p < 0.001$) reduced the TG level (121.45 ± 4.52 mg/dL) to near normalization and was comparable to the positive control (139.67 ± 2.32 mg/dL). Similarly, TC level significantly ($p < 0.001$) elevated (210.98 ± 3.45 mg/dL) in STZ induced diabetic rats as compared to the normal (93.45 ± 1.52 mg/dL). But, pre-treatment of BLHA 500 mg/kg significantly ($p < 0.001$) reduced the TC level (91.72± 1.09 mg/dL) to near normalization and was comparable to the positive control (Fig. 3A-B).

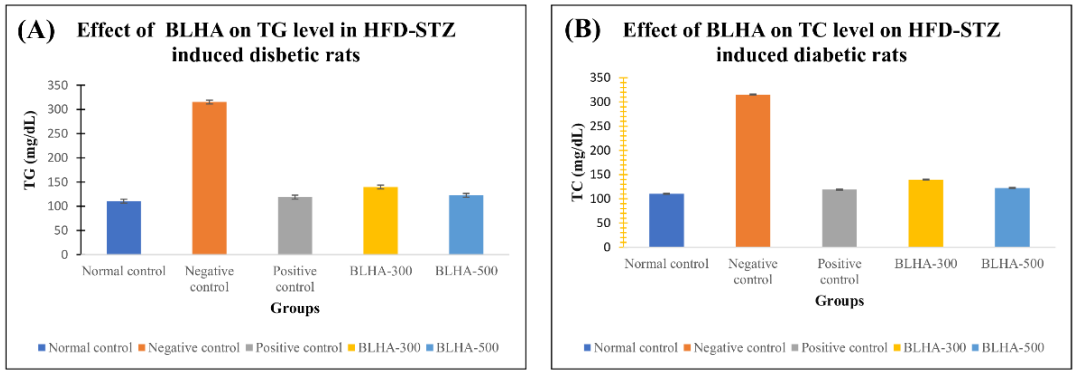


Fig.3. Demonstrating (A) Triglycerides level after BLHA administration. (B) Total cholesterol level after BLHA administration.

Establishment the renal functional markers:

In hyperglycemia, production of advanced glycated end product due to oxidative stress decreases the serum total protein (TP) level. Low serum TP content (Fig. 4A) was detected in diabetic rats (4.31 ± 0.63 g/dL) as compared to the non-diabetic rats (Fig. Similarly, in diabetic rats, there was a significant ($p < 0.001$) increase in the creatinine (Fig. 4B) concentration (2.52 ± 0.11 mg/dL) when compared to the non-diabetic rats.

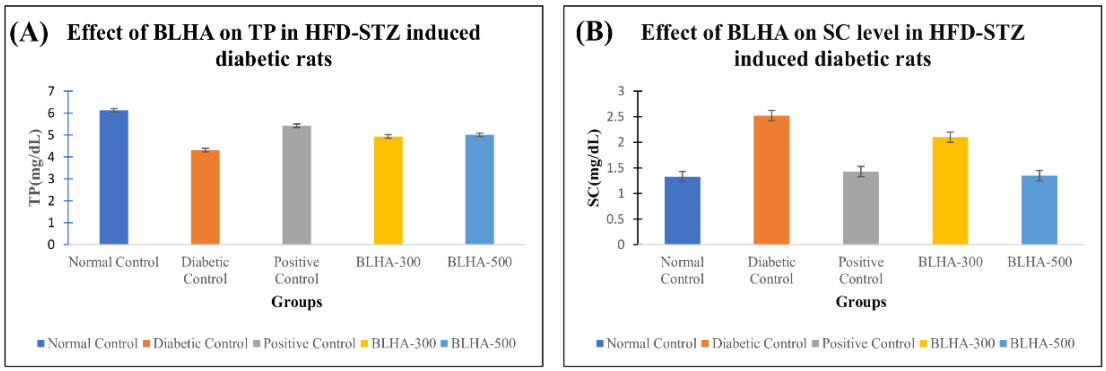


Fig. 4. (A) . Total protein level in diabetic rats after BLHA administration.(B) Serum creatinine level of diabetic rats after treated with BLHA extract.

Evaluation of Oxidative-stress markers of serum sample:

Our results demonstrated that negative control showed significant ($p < 0.001$) increase in MDA level in serum, liver and pancreas as compared to the normal. But, the pretreatment of BLHA-500 showed significant decrease in MDA level in serum (4.24 ± 0.08 nM/mg protein), and were comparable to positive control (Fig. 4A). The present study also analyzed the SOD level (Fig. 5A) significant ($p < 0.001$) decreased in serum, liver and pancreas in negative control as compared to the normal. But, pretreatment with BLHA-500 significantly ($p < 0.001$) increased the SOD level (serum 9.11 ± 0.21) and these results were found comparable to positive control (Fig.5B).

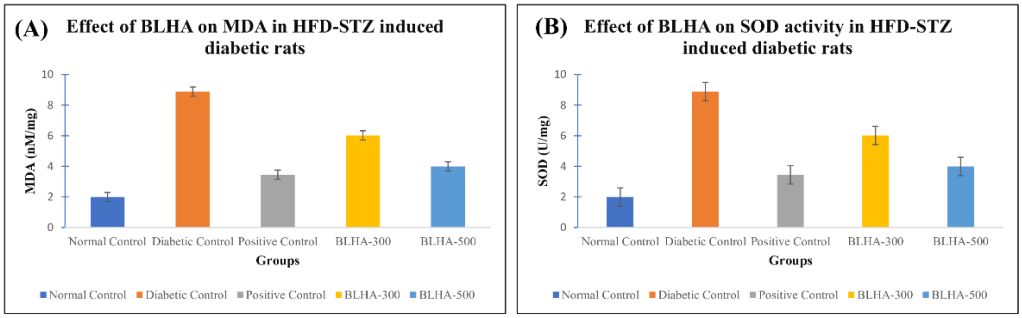


Fig. 5. (A) MDA level of diabetic rats after treated with BLHA extract.(B) SOD activity level of diabetic rats after treated with BLHA extract.

4.11 Development of Alternative regeneration method of rare mangrove species of Xylocarpus through vegetative propagation

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Xylocarpus is a genus of tree mangroves and characterized by its unique adaptations to saline and intertidal environments. These plants are found in coastal regions throughout Southeast Asia, the Indian subcontinent basically Sundarbans & Bhitarkanika, and parts of northern Australia. *Xylocarpus* species play a crucial role in maintaining the health and stability of mangrove ecosystems, which are invaluable for their ecological services, including coastal protection, carbon sequestration, and habitat for diverse flora and fauna. However, many species within this genus are currently classified as rare, endangered, or threatened due to a combination of factors, such as habitat loss, pollution, and climate change.

The conservation of rare, endangered, and threatened mangrove plant species in Odisha, such as *Xylocarpus*, is of paramount importance for maintaining biodiversity and preserving fragile ecosystems along with other associate species. *Xylocarpus* species, including *X. granatum, X. moluccensis, and X. mekongensis*, are critical components of coastal and mangrove ecosystems, which are under severe threat due to habitat loss and environmental degradation. However, natural recruit or regeneration is a serious concern for many tree mangroves including *Xylocarpus* spp. Hence, other alternative methods of propagation, especially macropropagation, required to be established for conservation of these threatened plants.

The Ecological Significance of *Xylocarpus:*

Xylocarpus species are essential components of mangrove ecosystems. They thrive in saline, waterlogged soils and are known for their unique adaptations to extreme environmental conditions. Their ecological significance can be summarized as follows:

Coastal Protection: The dense, intricate root systems of *Xylocarpus* species help stabilize coastal soils and protect against erosion caused by strong wave action and tidal currents.

Biodiversity Hotspots: Mangrove ecosystems, including those harboring *Xylocarpus*, support a rich diversity of plant and animal species. These areas serve as nurseries for numerous commercially and ecologically valuable fish species.

Carbon Sequestration: *Xylocarpus* trees store a significant amount of carbon in their biomass and play a vital role in mitigating climate change by sequestering carbon dioxide from the atmosphere.

Unique Adaptations: *Xylocarpus* species have evolved specialized adaptations for salt tolerance, including salt-excreting glands and pneumatophores, which allow them to thrive in high-salinity environments.

Challenges to *Xylocarpus* Conservation:

Despite their ecological significance, *Xylocarpus* species face a myriad of challenges:

Habitat Loss: Mangrove ecosystems are under constant threat from coastal development, aquaculture, and urbanization. These activities result in the loss of crucial *Xylocarpus* habitats.

Climate Change: Pollution from industrial and agricultural runoff, as well as oil spills, poses a severe threat to *Xylocarpus* and other mangrove species. Rising sea levels, increased temperatures, and extreme weather events linked to climate change can adversely affect *Xylocarpus* populations and their associated ecosystems.

Invasive Species: The introduction of non-native species can outcompete or displace *Xylocarpus* and other native mangrove plants.

Importance of Artificial Regeneration:

To address these challenges and ensure the continued existence of *Xylocarpus* species, artificial regeneration techniques are imperative. These techniques enable the controlled propagation of these plants in managed environments, thereby increasing their survival and restoring degraded ecosystems.

Vegetative Propagation:

Inadequate natural recruitment always limits sustainable population because of insufficient seed quality, poor seed germination & survival and poor reproduction by the next adult generation. In order to establish a viable and self-sustaining population in the wild, the plant individuals need to be augmented with re-introduction of vegetative propagated planting materials.

Air-layering: Air-layers of *Xylocarpus granatum, Xylocarpus mekongensis & Xylocarpus moluccensis* were produced through 'Air layering' as well as "Black taped Layering" using plant growth regulators (IBA, NAA, IAA) in pre-existed Mother plants at Bhitarkanika because it is an effective vegetative propagation method for the plants those do not root readily from cuttings. The success rate of re-production through Air layering in case of *xylocarpus spp.* is moderate.

Vegetative propagation at Bhitarkanika

Vegetative propagation in mangrove mother plant ***Xylocarpus granatum at*** Bhitarkanika

(22 44'3.63" N 86 52'7.69" E Dangmal & Kanika)

Vegetative propagation in mangrove mother plant ***Xylocarpus mekongensis at*** Bhitarkanika **(20 43'52.29" N 86 53'6.44" E Mathadia & Kanika (Inside**

Vegetative propagation in mangrove mother plant ***Xylocarpus moluccensis at*** Bhitarkanika **(20 42'24.72" N 86 52'01.37" E Sunopatia & Kanika)**

Then the hardening experiment was set under the shade-net house of RPRC, where the rooted leafy plants were allowed to grow in polypods with treatments of varied artificial NaCl-

Harvested rooted airlayers of *Xylocarpus* spp.

salinity following standard methods (Basak et al., 1995, 2000; Eganathan et al., 2000).Micro-stem cuttings (<10 cm long): juvenile shoots obtained from seedlings (to be raised in nursery conditions) as well as from mature branches of adult trees, was treated as micro-cutting explants; adventitious roots were generated following established methods using plant growth regulators (IBA, NAA, IAA) under mist using pre-girdled black-taping method. Vegetative propagation is an efficient method for rapidly multiplying *Xylocarpus* plants and preserving specific genotypes, making it a valuable tool for conservation efforts.

Plant efficiency and chlorophyll fluorescence activity analysis in *Xylocarpus* Species Grown in RPRC nuersery

Propagation Species	Mother Plant Description	Coordinates (Bhitarkanika)	Propagation Type	% of Rooting
Xylocarpus granatum	Height: 5-10meter Width: (0.3-2ft) Branches: Main 6-8 Bark: Smooth Leaf: Compound (3.5-12cm)	22 44'3.63" N 86 52'7.69" E Dangmal & Kanika	Air layering	85%
Xylocarpus mekongensis	Height: 8-15meter Width: (1-3ft) Dia Branches: Main 8-12 Bark: Flaky & Hard Leaf: Opposite leaflets(6-17cm)	20 43'52.29" N 86 53'6.44" E Mathadia & Kanika (Inside)	Air layering	35%
Xylocarpus moluccensis	Height: 10-17meter Width: 1-5ft) Branches: Main 8-12 Bark: Hard Leaf:2-3 leaflets & Egg shaped 4-12cm	20 42'24.72" N 86 52'01.37" E Sunopatia & Kanika	Air layering	Not respoded

Conclusion

The artificial regeneration of rare, endangered, and threatened species like *Xylocarpus* through vegetative methods has been a challenging effort. As an alternative method of seed propagation or natural regeneration , vegetative propagation methods were attempted for the mangrove plants *Xylocarpus granatum, Xylocarpus mekongensis & Xylocarpus moluccensis* . Various methods i.e., Air- layering, Black-taping, etc. were employed for better rooting & artificial regeneration using wild mother stock plant at Bhitarkanika as well as in ex-situ nursery-grown juvenile stock at RPRC. Growth & development of rooted layers were studied during hardening period using Plant efficiency analyser (PEA). Alternative methods i.e.air- layering found effective in *X. granatum* and *X. mekongensis* as far as rooting & sapling establishment are concerned. However, *X. moluccensis* did not respond in artificial rooting and acted as difficult-to-root species. For re-introduction, further mass propagation and hardening of saplings are in progress.

4.12 Immunity Boosting Natural Fruits: Determination of Ascorbic Acid (Vitamin-C) and Other Antioxidants for Selection of Potent Species to Promote Domestication.

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Wild edible fruits are considered as nutraceuticals since they offer both nutritional and potential therapeutic benefits. They are often packed with essential vitamins, minerals, and antioxidants. These nutrients can provide numerous health benefits when incorporated into one's diet.They may be regarded as natural immunity boosters to defend our bodies from numerous ailments because they are derived from natural sources. However, the decline in the ethno-medicinal knowledge of wild edible fruits due to lack of scientific records. Thus the use of palatable wild fruits is limited to the tribal, forest dwellers and villages who live adjacent to forests. In the 21st century food and nutritional security is a worldwide concern; addressing global food insecurity is a complex challenge, and it requires scientific and innovative approaches.Feeding in excess of 800 million under nourished people depend not only on increased productivity of limited number of domesticated crops of modern world but also the use of underutilized wild species to ensure sustainability.

The body's sophisticated immune system has the ability to identify foreign substances and neutralize, remove, or metabolize them without harming its own tissues. It is therefore crucial to have a strong immunity, and Vitamin-C (Ascorbic Acid, $C_6H_8O_6$) is helpful for this. Citrus and other fruits and vegetables are the main sources of vitamin C, a water-soluble micronutrient that is necessary for human health. It is also regarded as one of the most potent antioxidants, aiding in the prevention or postponement of some forms of cell damage as well as boosting immunity. They prevent oxidation, a chemical process that might result in free radicals and cascade reactions that could harm organisms' cells. It is widely marked as a dietary supplement for treating scurvy.

Antioxidants have great relevance in prevention and therapeutics of diseases. Natural antioxidants are essentially the plant secondary metabolites capable of slowing or inhibiting the harmful effects of free radicals and high levels of oxygen produced during photosynthesis. Various phytochemical and pharmacological studies strongly supported the fact that plants constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems for this reason of growing interest towards natural antioxidants of herbal resources is being develop gradually.

In current scenario of modern and synthetic medicinal era, use of some synthetic antioxidants has become curtailed due to their certain noxious effects along with increasing upsurge of interest in use of therapeutic potential medicinal plants as antioxidants has enlightened the path towards exploring new natural source for antioxidant compounds of plant origin diligently.Many studies have revealed that tropical fruits with high levels of vitamin C include lemons, oranges, jackfruit, guava, etc. However, reports suggest that low- and middle-income country citizens are deficient in vitamin C. This could be reduced by encouraging people to eat natural fruits with ascorbic acid levels close to those of cultivated fruits rather than taking supplements

because natural fruits also contain various antioxidants in addition to vitamin C. Thus, it is now more important than ever to domesticate natural fruits as immune boosters on a big scale.

In this context, the following objectives are taken into consideration for the study i.e. Isolation and quantification of Ascorbic acid (vitamin C) and other antioxidant properties in selected natural fruits in order to select the potent species to encourage its conservation and domestication as immune boosting natural fruits.

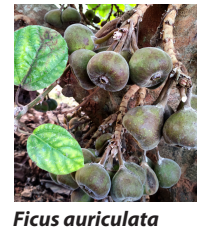
For this piece of work, the ten selected wild fruits species were collected from forest regions of Mid Central Table Land (MCTL) and East& South Eastern Coastal Plain (ESECP) regions of Odisha and preserved at -20% until further use.


Antidesma ghaesembilla


Artocarpus lakoocha


Carmona retusa


Carissa spinarunm

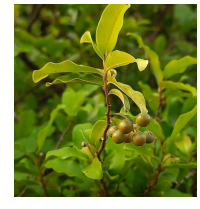

Ficus auriculata


Glycosmis pentaphylla


Limonia acidissima


Mimusops elengi


Phyllanthus acidus


Polyalthia suberosa

For the quantification of Ascorbic acid several methods have been used i.e. Volumetric, spectrophotometricand HPLC methods. Using the standard protocol by Al-Majidi and Al-Qubury, 2016 method followed by slight modifications and the OD was measured at 280nm using spectrophotometer (Analytik Jena, Spekol 2000, Germany).Reverse phase HPLC method with UV detection has been used (Rodriguez et al., 1992) for HPLC analysis performed in HPLC system (Thermo Fisher Scientific, Dionex™ Ultimate 3000) equipped with binary pump and porous silica with 5µm diameter C-18 column controlled by software Chromeleon™7.2.8.10783 version. HPLC analysis was performed by means of a C-18 column and a mixture of in acetonitrile and H_2O as mobile phase. The detection was done at 254nm. In order to select potent species further analysis of Antioxidant properties i.e. DPPH, SOD, CAT, POX,Flavonoid,Total Phenol, FRAP assay were carried out.It was observed that fruits collected from different locations shows different physiochemical changes. Along with this due to the variations in climatic conditions there is also a significant variation in fruiting period as well as in their chemical constitutions The studies have revealed thatfruits like *Phyllanthus acidus*(Mid central table land), *Carmona retusa(East and South Eastern Coastal Plain), Antidesma ghaesembilla(East and South Eastern Coastal Plain),Polyalthia suberosa(East and South Eastern Coastal Plain), Carissa spinarum(Mid Central Table Land), Limonia acidissima (East and South Eastern Coastal Plain)& Ficus auriculata(East and South Eastern Coastal Plain)* contains promising amount Ascorbic acid and other antioxidant properties which helps in preventing the initiation of chain reactions by removing free radicals, interrupting chain sequence , scavenging free radicals generated in chainreactions.

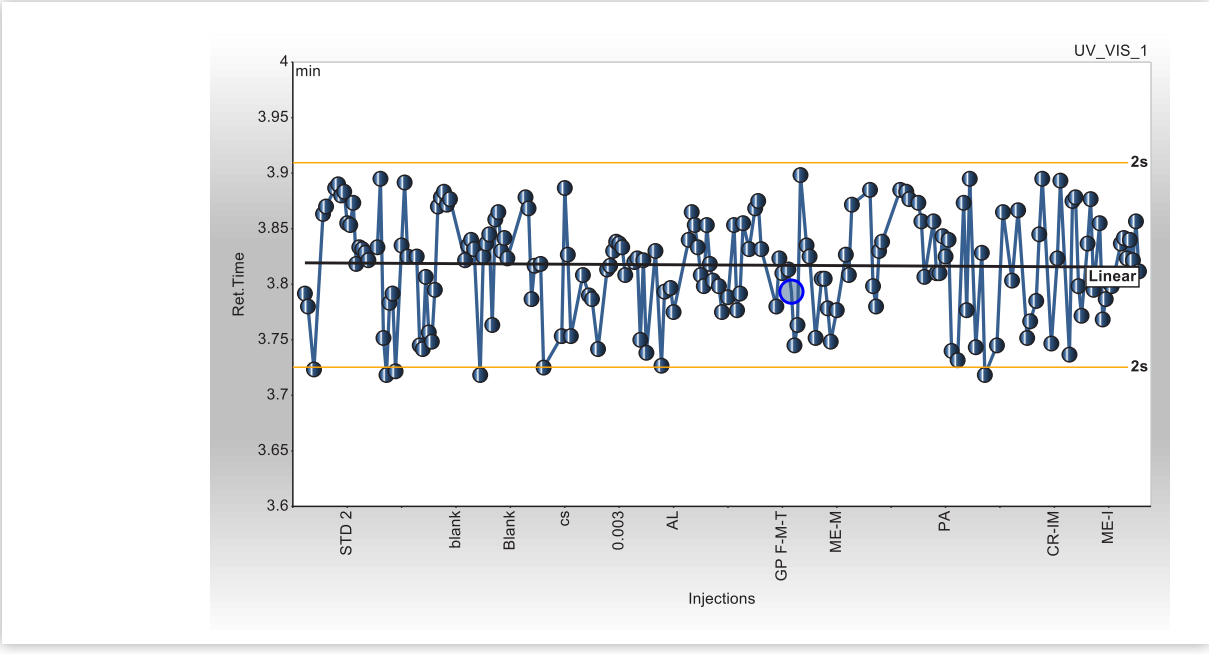


Fig 1: The above graph shows the retention time for analysisof L-Ascorbic Acid for specified fruits

Further, FTIR analysis aimed at characterizing and comparing the qualitative aspects of functional groups present in the dried fruit sample at its three stages of maturation collected from wild regions as mentioned. The similarity in the FTIR spectrum profile of three different stages of maturation of fruits provides fidelity of their phytoconstituents. The FTIR analysis indicated presence of different enriched functional groups i.e. C=O, N-H,C-H,O=C-O-C,C-C,C-O-C and –SCN among which L-Ascorbic Acid related functional groups were identified with reference to the standard spectral library. This determines the presence of Ascorbic Acid in the selected fruits species from which *Phyllanthus acidus, Antidesma ghaesembilla, Artocarpus lakoocha, Ficus auriculata & Limonia acidissima* were found to have very strong intensity bond of functional groups associated with L-Ascorbic Acid as mentioned above.Further study on these functional groups present in this particular fruit species, may lead to determination of important bioactive compounds that may open new avenue for pharmaceutical industry.It could thus be opined that these above wild edible fruits contains potent nutritional and antioxidant properties, thus should be popularized for consumption among the people in order to maintain good and healthy body and mind and domesticated for prevention of future food scarcity as well as benefit the stake holders of the state.

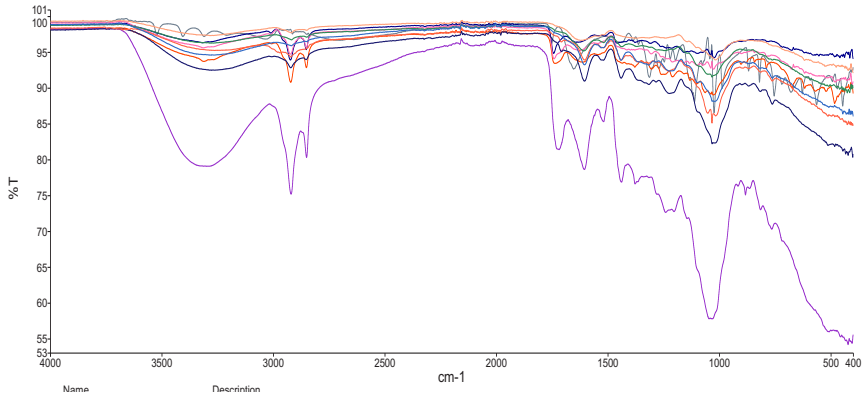


Fig2: ATR-FTIR spectrum for fruit samples

4.13 Field introduction and establishment studies of variously propagated wild edible fruits of Odisha

Principal Investigator: Dr. Uday Chand Basak, Senior Scientist

Conservation of wild edible fruit species is equally important as it helps to safeguard the genetic diversity of these species. This diversity is essential for the long-term survival of these plants and the sustainability of their ecosystems. The conservation of wild edible fruit species also ensures that their traditional knowledge and cultural practices are preserved for future generations. Additionally, the conservation of these species can have economic benefits by promoting sustainable livelihoods through the development of ecotourism and the sale of value-added products.However, the availability of wild edible fruits in Odisha is under threat due to various factors, such as deforestation, climate change, and overexploitation. Many of these fruits are also seasonal and have a short shelf life, making them difficult to transport and store. As a result, efforts are being made to conserve these fruits and promote their sustainable use. The wild edible fruits of Odisha are a significant natural resource that provides valuable nutrition and income for local populations. However, it is essential to conserve them and promote their sustainable use to ensure their continued availability for future generations.

Therefore, efforts to propagate and conserve these species are of utmost importance. Propagation of wild edible fruit species involves the process of multiplying these plants through various techniques such as grafting, cutting, and seed propagation. This is crucial for increasing the availability of these species and ensuring their sustainable production. Propagation also provides an opportunity for the improvement of these species through the selection of superior varieties and the development of new cultivars that are more productive, disease-resistant and adaptable to changing environments.The propagation and conservation of wild edible fruit species are critical for ensuring food security, preserving traditional knowledge, and promoting sustainable livelihoods. These efforts require the collaboration of various stakeholders, including governments, researchers, and local communities, to develop and implement effective strategies for the sustainable management of these species. Wild edible fruit species viz. *Antidesma ghaesembilla* (Nuniari) , *Carmona retusa* (Kujipana), *Eugenia rothii* (Sagadabatua), *Glycosmis pentaphylla* (Chauladhuakoli), *Polyalthia suberosa* (Guakoli) and *Toddalia asiatica* (Tundapoda) have been propagated and hardened under nursery conditions in RPRC and proposed for field establishment studies.Both seed and vegetative propagated saplings subjected to field introduction for generating comparative data on growth and development at their early stage of introduction in their natural habitat.

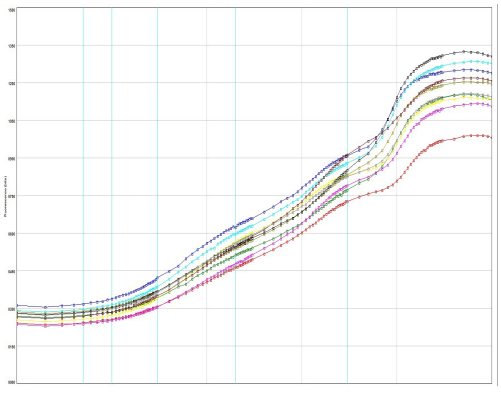
Growth and adaptability: Hansatech Handy PEA+ apparatus was used to analyze the maximum quantum yield (Fv/Fm) of Photosystem II and the Chlorophyll fluorescence Performance index (PI abs) in the propagated saplings.The data obtained were used to calculate two biophysical parameters that describe the photochemistry of PSII: maximum quantum yield of photosystem II (Fv/Fm) and performance index on absorption basis (PIabs).

During the study, Chlorophyll fluorescence of photosynthetic performance was analyzed. Measuring the Fv/Fm and PIabs parameters in photosynthesis of plants is crucial because they provide important information about the efficiency of photosynthesis and the health of the plant. Fv/Fm (variable fluorescence to maximum fluorescence ratio) is a parameter that indicates the maximum quantum efficiency of photosystem II (PSII) in leaves. It is a measure of how well the plant is able to use light energy for photosynthesis. A low Fv/Fm value indicates that PSII is not functioning properly, which could be due to damage or stress. Thus, monitoring the Fv/Fm ratio is an effective way to detect changes in PSII activity and diagnose problems related to photosynthesis in plants. PIabs (performance index calculated from absorption spectra) is another parameter that provides insight into the health of the plant. It was calculated from the absorption spectra of the leaves and reflects the efficiency of the light-harvesting complexes (LHCs) in transferring energy to the reaction centers of

PSII. A decrease in PIabs suggested that there is damage to the LHCs, and thus, the plant may not be able to capture enough light energy to carry out photosynthesis efficiently. Overall, measuring Fv/Fm and PIabs in photosynthesis of plants is necessary to monitor the photosynthetic efficiency and detect any stress or damage that could affect the growth and development of the plant.

The enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities were analyzed for the plants to help us understand the plant's ability to scavenge reactive oxygen species (ROS) and their adaptability to environmental stress. Proline content in plants accumulates in response to environmental stress, such as drought, high salinity, or extreme temperatures. Proline content was measured in the propagated saplings to access the level of stress a plant has experienced and evaluate its ability to adapt to changing environmental conditions.

Analysis of Performance index through Hansatech Handy PEA+ in the hardened saplings



#	Trace	Record No	ID	Date	Time	Fv/Fm	PI abs
1	✓	379		28/02/23	12:57:35	0.767	5.095
2	✓	380		28/02/23	12:57:58	0.762	3.652
3	✓	381		28/02/23	12:58:12	0.776	6.044
4	✓	382		28/02/23	12:58:32	0.789	4.901
5	✓	383		28/02/23	12:58:48	0.796	7.403
6	✓	384		28/02/23	12:59:02	0.782	5.371
7	✓	385		28/02/23	12:59:14	0.772	5.408
8	✓	386		28/02/23	12:59:28	0.791	6.184
9	✓	387		28/02/23	12:59:39	0.797	7.212
10	✓	388		28/02/23	12:59:52	0.769	4.776

Maximum quantum yield of photosystem II (Fv/Fm) and PIabs in the hardened saplings

Training and outreach program

During this year, the plants were established and grown in nursery conditions. Mass propagation and establishment study of wild edible fruit plants were carried out for further introduction of the species in wild habitat. In a nursery, plants are grown under controlled conditions, which may limit their genetic diversity. By introducing them into their natural habitat, have the opportunity to interact with other individuals of the same species, potentially leading to increased genetic diversity and stronger resistance to environmental stress. Introducing these plants into their natural habitat can help us to restore degraded ecosystems and promote biodiversity. During the 1st year of this project, site selection has been initiated (Chandaka Wildlife Division) for 1st phase introduction of 6 wild edible fruit species. However, the re-introduction work will be done in more other suitable sites as a part of continuation of this project work to obtain comparative data on growth and development of the introduced species to mark adaptability.

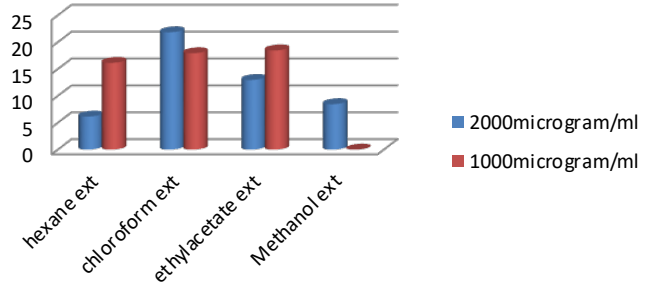
4.14 Assessment of antifungal activity of *Combretum roxburghii* and *Terminalia arjuna* solvent extracts against *Aspergillus flavus*.

Principal Investigator: Dr. Sunita Bhatnagar, Senior Scientist

Combretum roxburghii
Antifungal activity using radical growth method:

Antifungal activity was more on earlier days where as it reduced with passing time. Thus, it can be assumed that extracts had direct mode of action which diminished with the reduction of available extract in later days. Chloroform extract showed best biomass reduction amongst the solvents, where as radical growth was better in hexane extract.

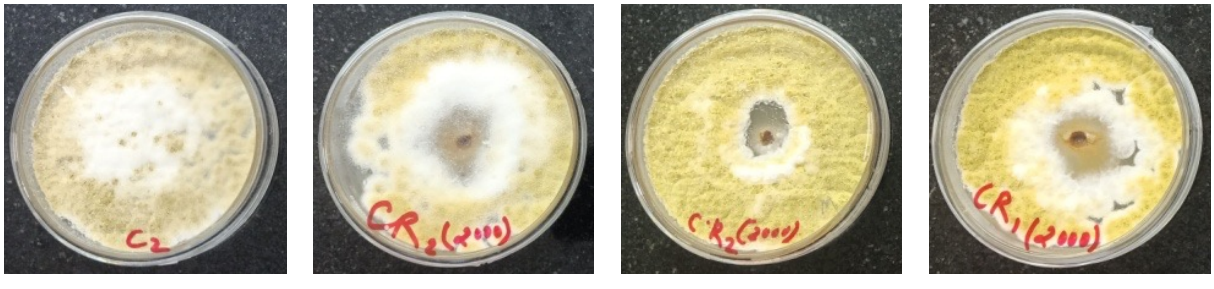
Fig . Biomass reduction after radical growth method.



Antifungal activity using agar well diffusion method

Except for hexane extract all the extracts showed significant zone of inhibition when compared with control

Fig : Zone of inhibition by solvent extracts of *Combretum roxburghii* at a dose of 2000microgram/ml.



Control | Hexane extract | Chloroform extract | Methanol extract

Isolation of aflatoxin from liquid medium and anti aflatoxin activity of solvent extracts.

Crude Aflatoxin was isolated by the liquid liquid separation as per the standard protocol. Percentage inhibition was calculated using weight of crude aflatoxin obtained and was compared with the control samples which were devoid of any extract. Similarly percentage inhibition was also calculated spectrophotometerically. Data is presented in Table 3, which shows remarkable inhibition in ethyl acetate extract. Thus, overall the same extract needs further exploration for its inhibitory potential against aflatoxin.

Table ; Percentage of inhibition in aflatoxin by solvent extracts of *Combretum roxburghii* extracts.

Sample	Dose	Weight of Crude aflatoxin (in gm)	% of inhibition	Absorbance	% of inhibition
Control		0.025		3.072	
Hexane	1000µg	0.015	40%	2.728	11.19%
	2000µg	0.013	48%	2.602	15.29%
Chloroform	1000µg	0.018	28%	0.838	7.61%
	2000µg	0.021	16%	2.481	19.23%
Ethyl acetate	1000µg	0.005	80%	2.436	20.70%
	2000µg	0.011	56%	2.728	11.19%
Methanol	1000µg	0.033	Nil	1.656	46.09%
	2000µg	0.034	Nil	2.227	27.91%

Terminalia arjuna

Radical growth and biomass reduction

Hexane and Chloroform extract exhibited 20% activity at a higher dose of 2000microgram/ml on day 2, later the activity declined. Remaining extracts were inactive. Biomass reduction was less than 10% in all the extract.

Antifungal activity of solvent leaf extracts of *Terminalia arjuna using radical growth method*

Sample	Dose (in µg)	Day-2		Day-4		Day-6		Day-8		Day-10	
		Growth (in cm) Mean	% inhibition ±SD	Growth (in cm) Mean	% inhibition ±SD	Growth (in cm) Mean	% inhibition ±SD	Growth (in cm) Mean	% inhibition ±SD	Growth (in cm) Mean	% inhibition ±SD
Control		2.37		4.38		6.68		8.14		8.40	
Hexane	2000	1.9	19.83	3.37	23.74	6.42	3.89	8.27	NIL	8.65	NIL
	1000	2.07	12.65	4.17	4.79	5.97	10.62	7.96	2.21	8.43	NIL
Chloroform	2000	1.88	20.67	4.45	NIL	6.22	6.88	7.9	2.94	8.55	NIL
	1000	2.31	2.53	4.5	NIL	6.54	2.09	8.25	NIL	8.9	NIL

Ethyl acetate	2000	2.28	3.79	4.68	NIL	6.411	4.04	7.51	7.73	8.22	2.14
	1000	2.53	NIL	4.8	NIL	6.73	NIL	8.05	1.10	8.55	NIL
Methanol	2000	2.25	5.06	4.42	NIL	6.25	6.43	7.67	5.77	8.35	0.59
	1000	2.05	13.50	4.1	6.43	6.25	6.43	7.75	4.79	8.26	1.66

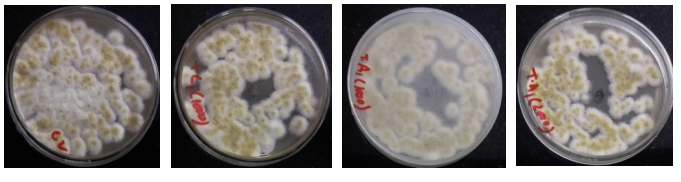
Antifungal activity using agar diffusion method:

In this method Methanol, ethyl acetate and chloroform extract showed zone of inhibition greater than 0.9 cm (Fig1) hence the extracts seem to inhibit the growth of fungus to a significant level. However in case of chloroform extract even positive control had zone of inhibition so the zone is due to the solvent hence same cannot be considered active. In case of ethylacetate and methanol extract there is a marked difference in positive control and the experimental samples, so the zone of inhibition is due to the extract not the solvent. Thus it can be concluded that study has provided lead for isolating antifungal principles from the above two extracts. Biomass reduction was not found significant.

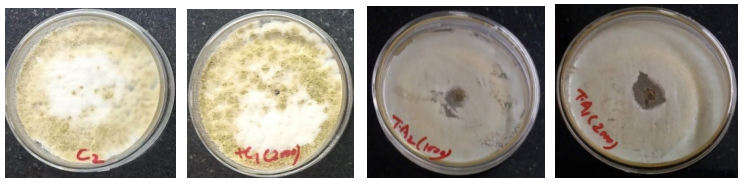
Antifungal activity of solvent extracts of *Terminalia arjuna* using zone of inhibition method					
Sl.no	Doses (microgram/ml	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1	1000	nil	1.3	0.67	1.9
2	2000	nil	1.1	0.91	1.15

Fig1. Zone of inhibition in solvent extracts of *Terminalia arjuna*

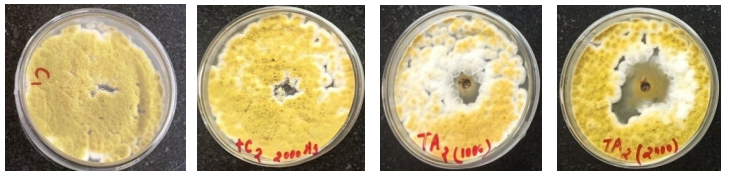
Chloroform extract



Ethyl acetate extract

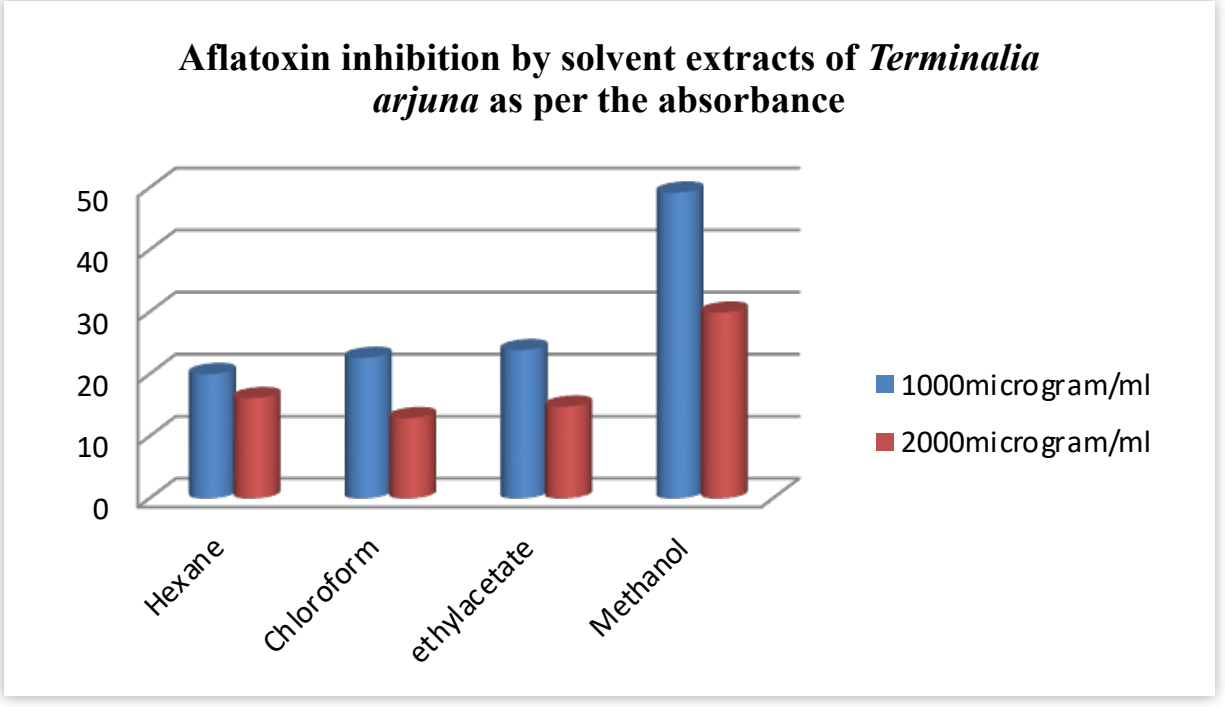


Methanol extract



Effect of extracts on biomass of fungus using liquid broth medium and aflatoxin isolation

As can be observed from Fig 2 that all the extracts showed inhibition in aflatoxin content when compared with the control. Maximum reduction was in methanol extracts but the action was not dose dependent. However biomass reduction was maximum in chloroform extract followed by methanol extract (Table 4). Aflatoxins are hazardous to the health of poultry[8] as well as human beings so a reduction of 50% in aflatoxin content is significant.



Biomass studies of solvent extracts of *Terminalia arjuna* in liquid broth media				
Dose (µg/ml)	Hexane	Chloroform	Ethyl acetate	Methanol
2000	NIL	91.12	NIL	41.17
1000	NIL	34.57	NIL	26.47

4.15 Evaluation of non viable seeds of Withania somnifera for biological activity

Principal Investigator: Dr. Sunita Bhatnagar, Senior Scientist

Withania somnifera is a cultivated crop, its seeds loose viability very quickly at room temperature. In the present study biological potential of non viable seeds was explored so as to put them for use in medicinal purpose.

Phytochemical analysis

Out of nine phytochemical, hexane extract showed the presence of flavonoids. In chloroform, ethyl acetate and methanol extract five secondary metabolites were observed which were flavonoid, saponin, tannin, cardiac glycosides and alkaloid(Table). 4

Table: Phytochemical Analysis of *Withania somnifera* seed extracts.

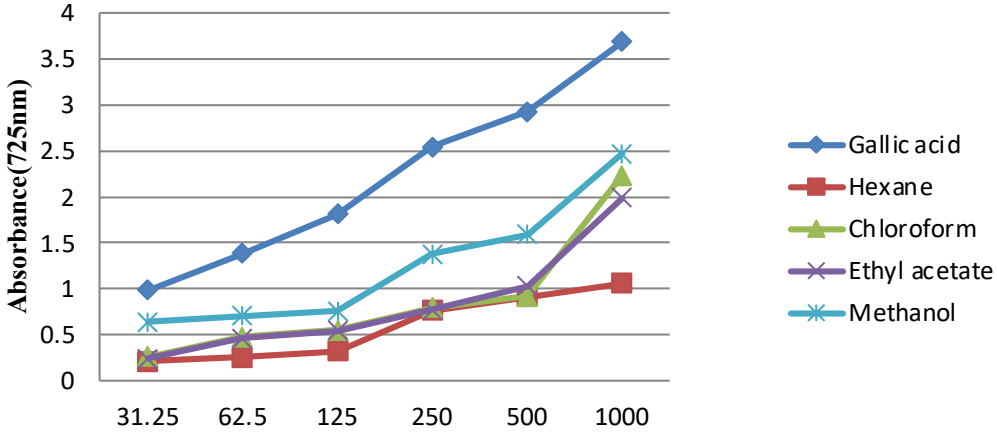
Phytochemical	Hexane	Chloroform	Ethyl acetate	Methanol
Flavonoid	+ ve	+ ve	+ ve	+ ve
Anthraquinone	- ve	- ve	- ve	- ve
Saponin	- ve	+ ve	+ ve	+ ve
Tannin	- ve	+ ve	+ ve	+ ve
Terpenoid	- ve	- ve	- ve	- ve
Phlabotanin	- ve	- ve	- ve	- ve
cardiac glycoside	- ve	+ ve	+ ve	+ ve
Starch	- ve	- ve	- ve	- ve
Alkaloids test	- ve	+ ve	+ ve	+ ve

+ indicates presence of phytochemical, whereas - indicates absence of phytochemical .

Determination of total Phenolic content:

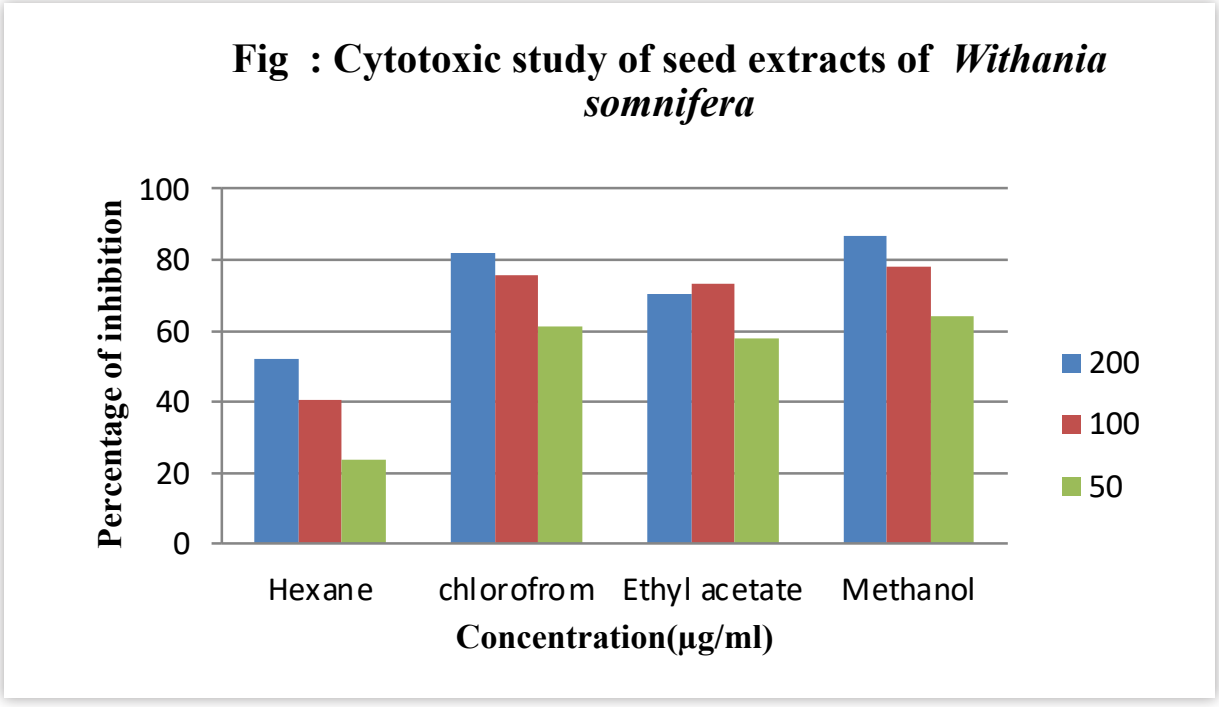
Highest concentration of phenolic was found in the methanol extract of the plant i.e. Methanol extract showed highest phenolics content followed by Ethyl acetate and chloroform. Phenolic are basically polar molecules hence presence of same in polar extracts is reasonable.

Fig : Phenolic contents in different solvents extracts of *Withania somnifera*



Cytotoxic activity using brine shrimp assay

Cytotoxic activity was found highest in methanol extract (86.88 %) at the dose 200µg/ml followed by chloroform which showed 81.81% activity at higher dose. Remaining extracts showed mild activity against brine shrimp mortality assay . Except for ethyl acetate extract all the other extracts showed dose dependent activity.

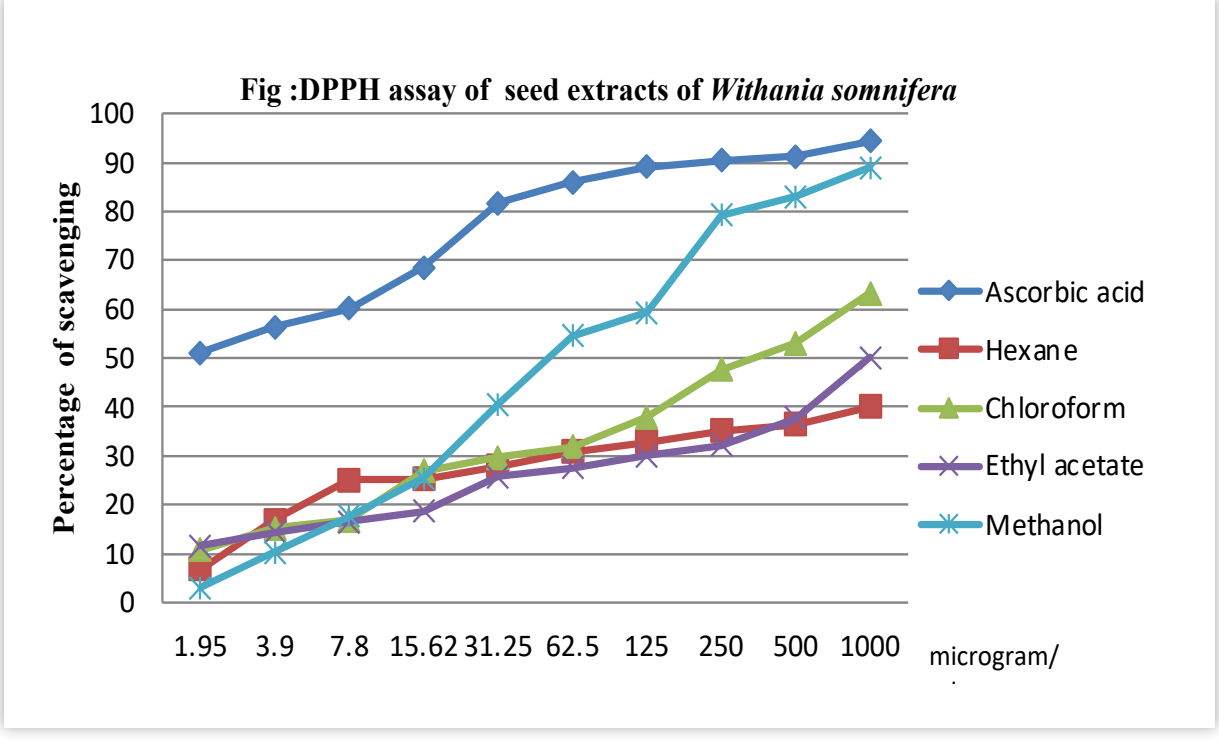


Antioxidant activity of extracts of Withania somnifera

Quantitative Antioxidant Activity

DPPH Free Radical Scavenging Assay

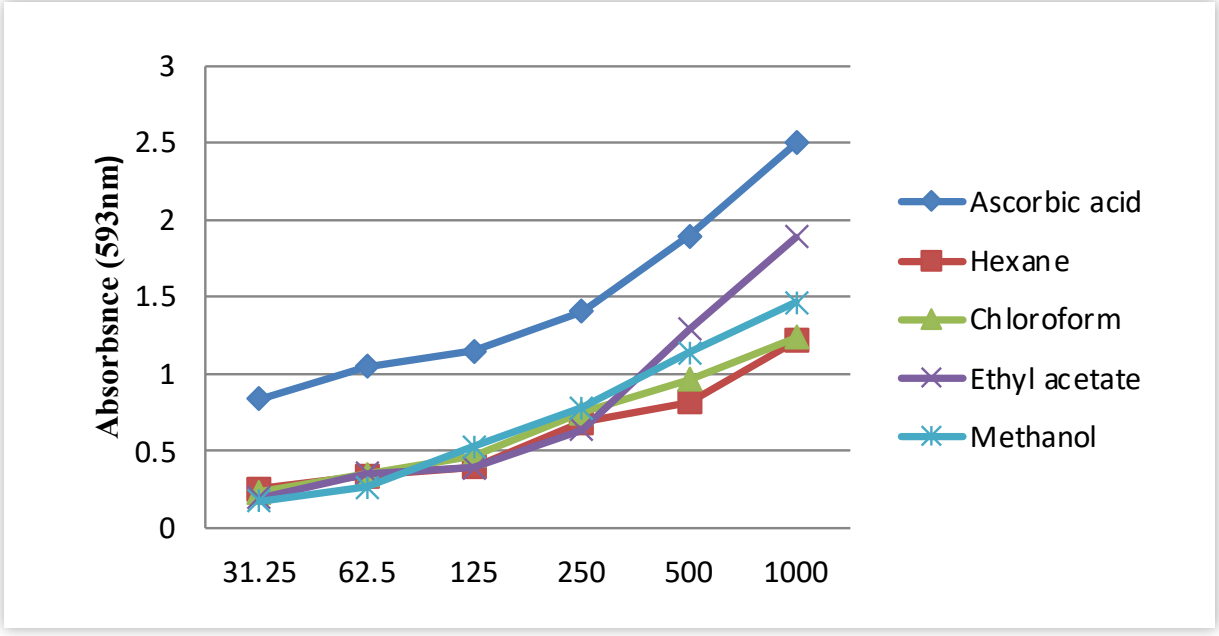
As can be observed from Fig below, Methanol extract showed highest activity of about88.91% where as hexane extract exerted an inhibition of 40.06%, Chloroform extract exerted an inhibition 63.30%, ethyl acetate extract exerted an inhibition of 50.10% at a higher dose of 1000microgram/ml.



Ferric reducing antioxidant power assay:

As can be observed from Fig , all the extracts showed less activity in the assay as compared to the standard which is a pure molecule which is a pure molecule whereas extracts are mixture of large number of molecules.

Fig : Ferric reducing ability of different extracts of *Withania somnifera*



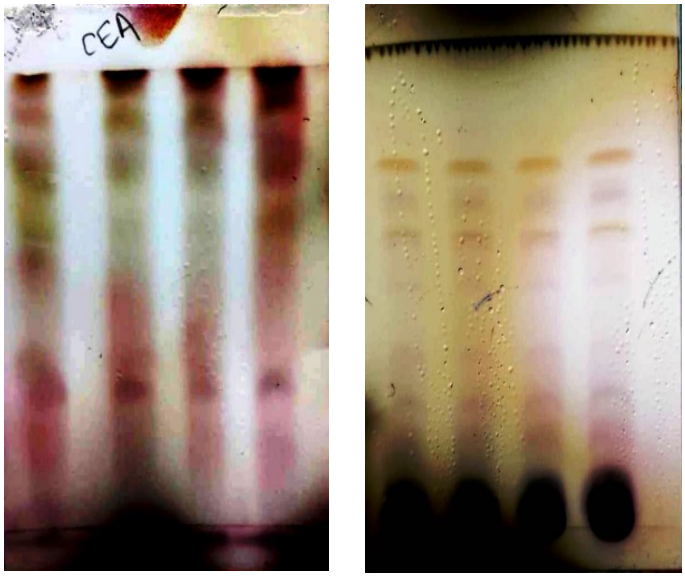
Assessement of withanolides in seed extracts of *Withania somnifera*.

Out of five solvents only 3 solvents showed separation in extracts and as can be seen from TLC slides extracts of non viable seeds also possessed withanoloides.

Table :TLC based analysis of seed extracts of *Withania somnifera* for analysis of withanoloides and other molecules.

Sl no.	Solvent system	Rf Values							
		Hexane		Chloroform		Ethyl acetate		Methanol	
		Rf value	No. of band	Rf value	No. of band	Rf value	No. of band	Rf value	No. of bands
1.	Toluene :Ethyl acetate: Acetic acid(60 :40:4)(TEA)	0.34,0.66, 0.73,0.8	4	**0.18,**.2, 0.62,0.71	4	**0.23,** 0.52, 0.65, 0.7, 0.79	5	0.28,0.51, 0.63,0.67, 0.75, 0.8	6

3.	Chloroform :Ethyl acetate: Methanol :Toluene (72 :4 :8 :16) (CEMT)	0,24, 0.3, 0.4,**0.5**, 0.55,0.62	6	0.17,0.26, **0.5**, 0.58	4	0.1, 0.24, 0.2, 0.49, 0.55, 0.6	6	0.19, 0.2, 0.3, **0.33**,0.38,0.42.0.46, **0.51** ,0.55,0.6	10
4.	Chloroform : Methanol :Water (64 :50 :10) (CMW)	0.25, 0.34, 0.78, 0.65	4	0.27, 0.55, 0.66, 0.75, 0.79	5	0.28,0. 67,0. 78,0.8	4	0.26,0. 51,0,7 ,0.65,0. 76,0.8	6



1= Withaferin A ; 2= Withanolide;3= Withanolide D; 4= Withaferin A

4.16 Propagation and reintroduction of selected endangered species of Odisha

Principal Investigator: **Dr. Kalidass, C. Scientist**

Seed morphology of *Oroxylum indicum* (L.) Kruz.:

Oroxylum indicum (L.) Kruz. is a medicinal plant used for treating various ailments such as stomach pain, ulcers, rheumatism and many more. It is crucial to conserve and protect the endangered species of medicinal plants like *Oroxylum indicum*. Therefore, seeds from various localities were collected to study their biology and germination. To break the seed dormancy, physical and chemical treatments were used, including scarification, hot and cold-water treatments and the use of plant growth regulators. A total of 50 mature *Oroxylum indicum* trees were selected from different parts of Odisha to collect their fruits. The seeds were collected in different seasons and their length, width, thickness and weight were measured and depicted in Fig. 1a & 1b.

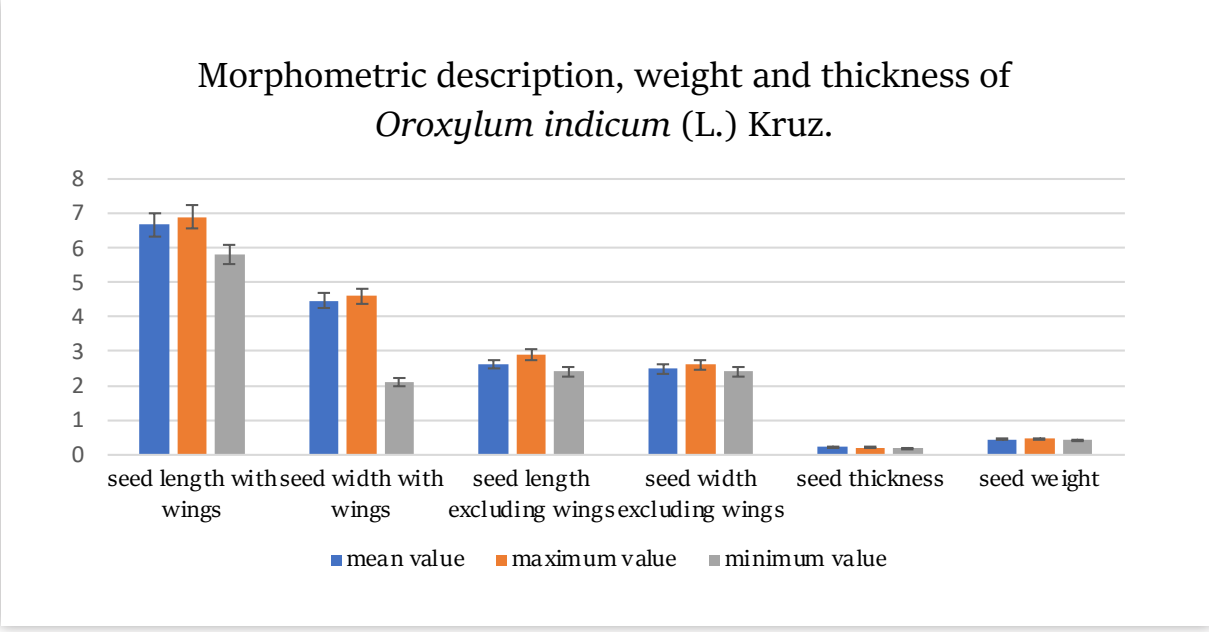


Fig. 1a. Morphometeric descritption, weight and thickness of *Oroxylum indicum* (L.) Kruz. Seeds

Fig. 1b. A view of mature fruits of *Oroxylum indicum* (L.) Kruz. Seeds.

The experiment involved a 90-day reading of seeds both with a control group and with the use of different growth hormone. Initially, the seeds that were treated and the control group did not respond until the 5th day, but on the 10th day, we observed two seeds germinating which were treated with growth hormone. As the number of days passed, the results for the seed sample treated with growth hormone increased gradually. On the 30th day, the treated seeds had germinated to 392, whereas the control group had only produced 17. By the 60th day of the experiment, the control group had only 35 seeds germinating, whereas the seeds treated with growth hormone responded with 930 germinations.

Finally, on the 90th day, when we summed up our calculations, the control group had a total of 47 germinations, while the treated seedlings had a total of 2000 germinations. Our experiment showed that the seeds treated with plant growth regulators gave immense results compared to those without the hormone within the same time period. The effect of plant growth regulators, such as GA_3, temperature regimes and photoperiodic conditions for synchronization of uniform germination in *Oroxylum indicum* was significantly successful Fig.2.

Fig. 2. *Oroxylum indicum*: a. Fruits, b. Winged seed, c. Unwinged seed, d. Radicle initiation, e. Unwinged, f. Shoot initiation, g. Different stages of seedling, h. germination in sand media, i. Shoot elongation, j. Elngated shoot in polybag condition, k. Seedling are ready for the reintroduction stage.

Reintroduction of *Oroxylum indicum*

The purpose of this study was to provide information on how to prevent harmful effects of inbreeding and outbreeding and preserve the natural genetic pattern of *Oroxylum indicum*. This information can be used to maximize the effectiveness of future population restoration and reinforcement. However, it should be noted that each reintroduction project is specific to the species involved, making generalizations difficult. Nevertheless, our research can be used to make inferences about how to successfully reintroduce *Oroxylum indicum*. The goal of macro-propagation in ex-vitro conservation is to limit plant species hybridization. It has been shown that employing plant material from big, stable source populations can increase the effectiveness of reintroductions. However, in the case of *Oroxylum indicum*, large populations were not always the genetically most variable. Therefore, plant material from 50 to 200 individuals belonging to various age and size classes who were present in the same location should be collected from these groups wherever possible. The selected endangered medicinal plant species has been restored in identified geographical areas within the state of Odisha. *Oroxylum indicum* has been established in two viable populations of 550 individuals each, for a total of 1100 individuals in Bargarh and Khordha district of Odisha. These populations are expected to strength the overall population and prevent inbreeding in the near future.

Due to their significant medicinal value, the plants have been preserved in the naturally expanding population. Although combing plant material from several source populations has been used effectively, this method should be used with caution because of the possibility of outbreeding depression, which can lower fitness and performance. We strongly advise against utilizing multiple source populations for reintroduction and population reinforcement since *Oroxylum indicum* displayed a highly different spatial pattern of genetic diversity. Instead, if genetic variation makes reintroducing the species necessary, we advise a gradual approach. Careful management of the reintroduction sites improves the success of the reintroduction, and a trustworthy and ongoing monitoring system enables the assessment of population reintroduction success.

Fig.3. *Oroxylum indicum* (L.) Kruz. Mass-propagated plants in polybags under controlled conditions for reintroduction / restoration in the same agro-climatic regions in Odisha. Various activities, such as the application of a nutritional mixture, plantation involving people and researchers were conducted.


OR 02BV 2095


Regional Plant Resource Centre
Forest, Environment and Climate Change Department,
Govt. of Odisha
Name of the Project : "Propagation and reintroduction of selected endangered species of Odi
(State Plan Project)
Co-ordinators : Smt. Pusazhule Mekro, IFS, PCCF & Chief Executive,
PI : Dr. Kalidass. C. Scientist
Reintroduction of Endangered Flowering Plants
Name of the Plant species : Oroxylum indicum (L.) Kurz.
Name of the Family : Bignoniaceae
No. of Seedling : 500 numbers
Location : Khordha District
Year : 2022

Oroxylum indicum: Reintroduction in Tangi Forest Range, Khordha Dist., Odisha.


Latitude: 19.962509
Longitude: 85.333802
Elevation: 80.01±7 m
Accuracy: 5.5 m
Time: 26-07-2022 10:19
Note: Dr Kalidass C


Latitude: 19.962399
Longitude: 85.333874
Elevation: 64.72±4 m
Accuracy: 7.6 m
Time: 26-07-2022 16:09
Note: Dr Kalidass C


Regional Plant Resource Centre
Forest, Environment and Climate Change Department,
Govt. of Odisha
Name of the Project : "Propagation and reintroduction of selected endangered species of Odisha"
(State Plan Project)
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PI : Dr. Kalidass. C. Scientist
Reintroduction of Endangered Flowering Plants
Name of the Plant species : Oroxylum indicum (L.) Kurz.
Name of the Family : Bignoniaceae
No. of Seedling : 500 numbers
Location : Khordha District
Year : 2022
Latitude: 19.962359
Longitude: 85.333764
Elevation: 76.43±3 m
Accuracy: 12.2 m
Time: 26-07-2022 15:31


Regional Plant Resource Centre
Forest, Environment and Climate Change Department,
Govt. of Odisha
Name of the Project : "Propagation and reintroduction of selected endangered species of Odisha"
(State Plan Project)
Co-ordinators : Smt. Pusazhule Mekro, IFS, PCCF & Chief Executive,
PI : Dr. Kalidass. C. Scientist
Reintroduction of Endangered Flowering Plants
Name of the Plant species : Oroxylum indicum (L.) Kurz.
Name of the Family : Bignoniaceae
No. of Seedling : 500 numbers
Location : Bargarh District
Year : 2022
Latitude: 21.227392
Longitude: 83.245977
Elevation: 263.38±5 m
Accuracy: 11.7 m
Time: 14-07-2022 13:43
Note: Dr Kalidass C
Powered by NoteCam

Seed morphology of *Pterocarpus santalinus* L.f.:

Pterocarpus santalinus seeds generally have a hard seed coat and have been reported to show improved germination rates with different pre-sowing and chemical treatments. Table 1. Summarizes the physical parameters of the seeds, including seed length with and without wings, seed thickness, seed width and collection period. After analysing the data of at least 10 randomly chosen seeds, we inputted the information into the system to evaluate the impact on the germination process.

Table 1. Morphological traits of seed of *Pterocarpus santalinus* L.f.

Seed with wings	Seed without wings	Seed thickness	Seeds width
2.7	1.8	5.2	5.0
2.9	2.0	5.3	5.2
3.2	2.9	6.0	5.5
3.1	2.7	5.1	4.1
6.1	5.8	4.9	4.5
4.2	3.8	5.0	5.9
5.8	4.9	4.6	5.7
3.7	3.4	5.5	4.7
3.9	3.5	5.7	5.1
4.0	3.6	6.1	4.8
Mean for 10 seeds- 39.6	34.4	53.4	50.8
Average- 3.96	3.44	5.34	5.08

Seed germination of *Pterocarpus santalinus* L.f.:

Germination started on 20th day with only three seeds. Swelling of the seeds was noticed on the 17th day. The maximum number of germinations was observed on the 45th day, followed by a decrease in germination that was not recorded. It can be concluded that no germination was observed on the 50th day. A total of 62 seeds germinated during the two-month duration of the experiment, while 6.8 seeds remained fresh and un-germinated (Table 2). Upon examine Table 3, it can be observed that the sum of live replicates with 100 seeds was 1.645g, while the average weight was recorded to e 0.329g. Based on these values, the weight of five hundred seeds was estimated to be 3.29g. The range value was assessed by subtracting the largest seed weight from the smallest seed weight among the five replicates studied with different treatments. The estimated standard deviation was found to be 0.030g, while the variation coefficient was recorded to be 0.93.

Table 2. 500 seeds at different GA_3 treatment with days of germination (Germination Percentage)

GA_3	Days of Germination										
	10	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**Response**	**Fresh not germinated**
1000ppm	0	0	0	2	2	2	4	10	0	8	2
2000ppm	0	0	0	10	10	10	10	40	0	34	6
3000ppm	0	0	0	10	20	30	30	90	0	82	8
4000ppm	0	0	10	20	30	40	50	150	0	143	7
5000ppm	0	0	5	10	5	0	0	20	0	9	11
Percentage	0	0	3	8.4	13.4	16.4	18.8	62.00	0	55.2	6.8

Average X= 0.329(n=5)

Weight of 500 seeds= 3.29gm

Range= Largest- smallest= 0.450mg-0.219 mg= 0.231g

Estimated Standard Deviation= 0.231/7.53= 0.030g

Var. of Co-eff. = 100× S.D/X= 100×0.030/3.29= 0.91g

Table 3. 500 seeds treated with different proportion of GA_3 for seed weight evaluation

Replicate	Number of seeds	GA_3 Treatment	Weight in mg
1	100	1000	219
2	100	2000	320
3	100	3000	356
4	100	4000	450
5	100	5000	300

We tried different methods to propagate seeds, as several factors are responsible for good germination, with temperature being one of the most important. Since most seeds germinate best at an optimum temperature that is usually higher than most home night temperatures special warm areas must often be provided. The use of thermostatically controlled heating cables is an excellent method of providing constant heat. We treated the seeds with both hot and cold water and later transferred them to a sand bed where the optimum temperature was slightly lower than the surrounding environment. The same seeds were then treated with different concentrations GA_3 [1000 – 4000 ppm]. Based on the results, it can be concluded that the seeds responded well to GA_3 treatment of 4000 ppm in 24hrs (Fig.1a &1b). we also treated the seeds with 5000 & 6000 ppm, but the seeds did not respond well enough. Therefore, we planted the seeds treated with 4000ppm, which resulted in 60% germination (Table 4).

Table. 4. Efeects of physical treament and plant growth regulators in seed germination of *Pterocarpus santalinus* L.f.

Sl No	Normal seed weight (gm)	Cold water treatment (gm)	Hot water treatment (gm)	GA3 Treatment [24hrs]			
				1000 ppm	2000 ppm	3000 ppm	4000 ppm
1	2.5	2.7	2.8	2.8	2.9	2.9	3.2
2	2.4	2.6	2.6	2.6	2.8	2.8	3.3
3	2.6	2.8	2.9	2.9	3.0	3.0	3.0
4	3.2	3.4	3.4	3.4	3.5	3.5	4.3
5	3.0	3.5	3.5	3.5	3.6	3.5	3.9
6	2.9	3.2	3.3	3.3	3.3	3.3	3.6
7	3.2	3.4	3.4	3.4	3.4	3.4	3.8
8	3.4	3.5	3.6	3.6	3.6	3.6	3.9
9	2.9	3.4	3.5	3.5	3.5	3.5	4.0
10	3.2	3.4	3.4	3.4	3.4	3.4	4.0

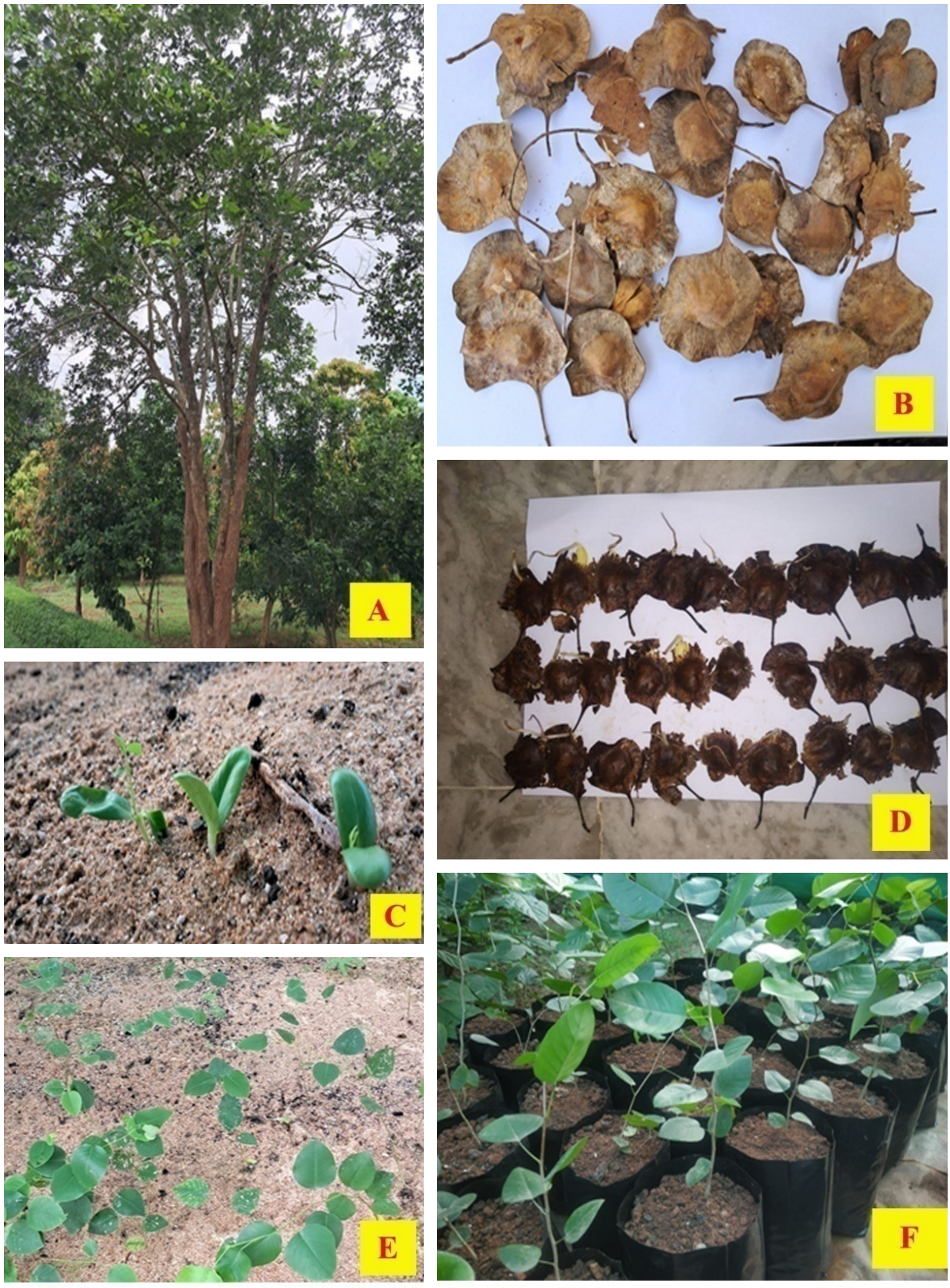


Fig. 1a: Pterocarpus santalinus L.f., A view of normal seed materials for seed germination with different treatment and breaking the dormancy of germinations.

Seed germination of *Cryptocarya amygdalina* Nees.:

The morphological data of the seeds was recorded on the basis of seed length, seed width and seed thickness before the chemical treatment of the seeds. The average data was then calculated for each of the characteristics, which were found to be 1.494 cm, 1.058 cm and 1.018 cm respectively. Some seeds were taken as control without any chemical treatment and more than half of the remaining seeds were treated with different concentrations of GA_3 [1000 – 5000ppm] with different time durations of about 24, 48 and 72hrs.

According to our experiment, the seed germination started first on 1000 ppm of GA_3 that was soaked for about 24hrs. the seeds responded on the 31st day of preliminary treatment after the seed was sown, whereas the control treatment took the required maximum time and initiated its germination on the 66th day. The germination percentage was calculated to be 25.39%, which was the lowest germination recorded. In contrast, seed treatment with GA_3 of 1000 ppm for 48 and 72hrs showed germination rates 53.92 and 52.65%, respectively. Therefore, it could be concluded that the minimum germination period was between 30-40 days.

Fig. A view of seeds of *Cryptocarya amygdalina* Nees., T.S. of endosperm with healthy embryo and a few seed germinations in sand bed

Seed germination of *Alphonsea madraspatana* Bedd.:

The population size of *A. madraspatana* has significantly declined over the years and currently, only a few isolated populations of the species remain in its natural habitat. It has also observed that the plant has a low reproductive output and limited genetic diversity, which could further threaten its survival in the long run. Based on the above views, it is under immediate threat and urgent action is require to conserve and protect endangered *Alphonsea madraspatana* plants. We have been collected seeds from Khandagiri and Udaygiri forest, Bhubaneswar and started the seed germination process for mass propagation. The preliminary investigated has been conducted with water soaking treatment, which has resulted in a few number of seeds being germinated. Form the initial experiment, it has been observed that the plant has various stages of seed germination such as radicles appearing in seed germination, a group of seedling and the grow of 30 plants that are being kept in good condition in polybags. [Fig. 1a&b]. Secondly, we are planning to conduct mass propagation methods such as rooting stem cutting and air-layering.

Because they can't bloom in time, ***Litsea glutinosa*** and ***Cordia macleodii*** might not produce seeds. We may conclude that alternative methods for mass multiplication must be pursued since we were unable to conduct the seed germination experiment on these plant species.

Fig. 1a. *Alphonsea madraspatana* Bedd. A. A view of habitat, B. Fruits, C. Mature fruit, D. Seeds

Fig. 1b. A view of seedling with measurement with different stages of *Alphonsea madraspatana* Bedd.

4.17 Standardization of *in vitro* regeneration techniques in red banana and establishment of red banana in Odisha climate condition

Principal investigator: **Dr. Kalidass, C., Scientist**

Tissue culture banana or micropropagation of banana is an important and sigificant technique used for the large-scale production of banana plants. It involves the propagation of plants using tissue culture techniques, allowing for the mass production of disease-free and genetically identical plants. One of the main benefits of tissue culture banana is the ability to rapidly prodcue large numbers of healthy plants with predictable characteristics. This method also allows for the production of plants that are resistant to diseases and pests, which is crucial in the banana industry since bananas are highly susceptible to various diseases. It is also important for the preservation of banana biodiversity. The banana industry has a limited number of commercially important cultivars and tissue culture techniques allow for the preservation and propagation of rare or endangered cultivars. In addition to its commercial benefits, tissue culture banana is also valuable for research purposes. It allows for the study of plant growth and development in a controlled environment, which can lead to a better understanding of plant physiology and genetics.

The shoot tip explants of red banana were subject to various surface sterilizing agents with different concentrations and time intervals. The most effective treatment involved treating the explants with 0.1% mercury chloride for 6 min, resulting in a 98% survival rate and contamination-free cultures. After one week of inoculation in initiation media, the shoot tip explants appeared externally as light brown in colour, but later turned greenish and developed adventitious shoots. The highest percentage of shoot establishment was obsevred in MS+ BAP treatment. Red banana explants inoculated in MS medium supplemented with BAP showed the highest number of pro-embryos/ explants [14.5±1.04] (**Fig.2**), while MS media Kinetin showed the least amount of pro-embryos/ explants [2.25±0.28] (**Table 1**). The maximum average number of shoots [6.86] was obtained on MS medium supplemented with BAP + IAA +ADS on the 6th subculture. The longest average on MS medium supplemented with BAP in addition to IAA and ADS 15o mg/l on the 6th subculture (**Table 2**).

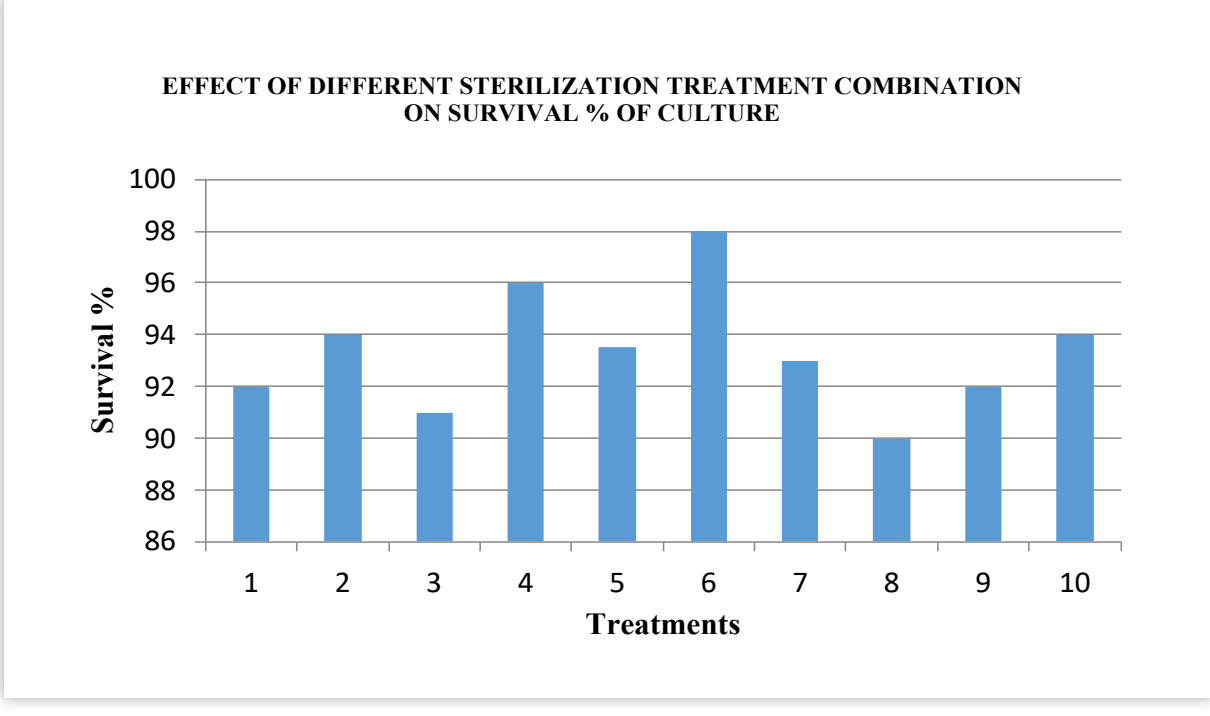


Table 1: Effect of plant growth regulators with different concentrations on shoot establishments of red banana

Treatments	PGRS	No of Pro-embryo /explants				mean
T1	BAP	5	3	8	1	4.25±1.80
T2	BAP	9	7	8	13	9.25±1.60
T3	BAP	2	10	3	1	4.00±2.36
T4	BAP	11	15	18	14	14.50±1.04
T5	Kinetin	8	1	0	0	2.25±0.28
T6	Kinetin	4	3	6	2	3.75±1.04
T7	Kinetin	7	5	8	7	6.75±0.76
T8	Kinetin	10	14	13	7	11.00±1.89

Both BAP and kinetin are cytokinins, plant growth hormones that promote cell division and shoot formation. In vitro studies have shown that both BAP and kinetin can be effective in promoting shoot establishment in banana micro-propagation. However, the effectiveness of each cytokinins may depend on the specific cultivar of banana and the composition of the growth medium used. Several in vitro studies have compared the effects of BAP and kinetin on shoot establishment in banana micro-propagation. One study reported that BAP was more effective than kinetin in promoting shoot proliferation in banana cv. Rasthali. Another study found that kinetin was more effective than BAP in promoting shoot proliferation in banana cv. Red ABB. Generally, the choice of cytokinins for shoot establishment in banana micro-propagation may depend on several factors such as the cultivar being propagated, the desired growth characteristics and the composition of the growth medium. It is important to note that the optimal concentration of BAP or kinetin may also vary depending on these factors.

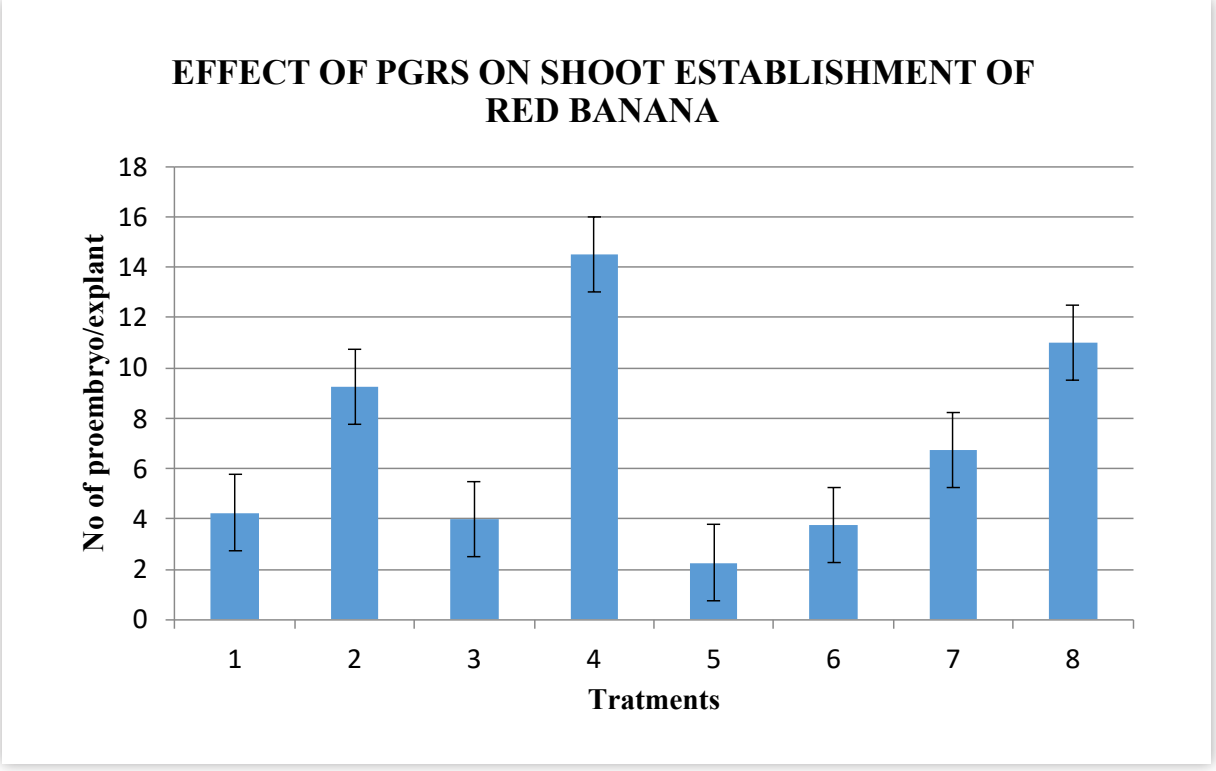
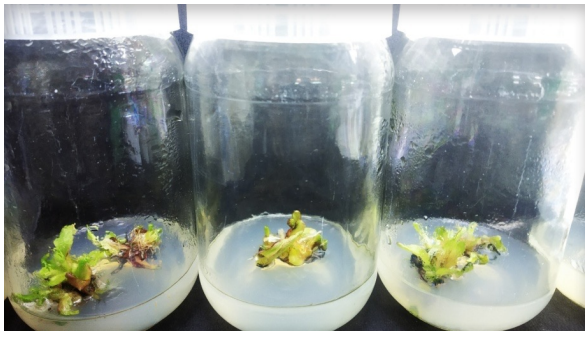
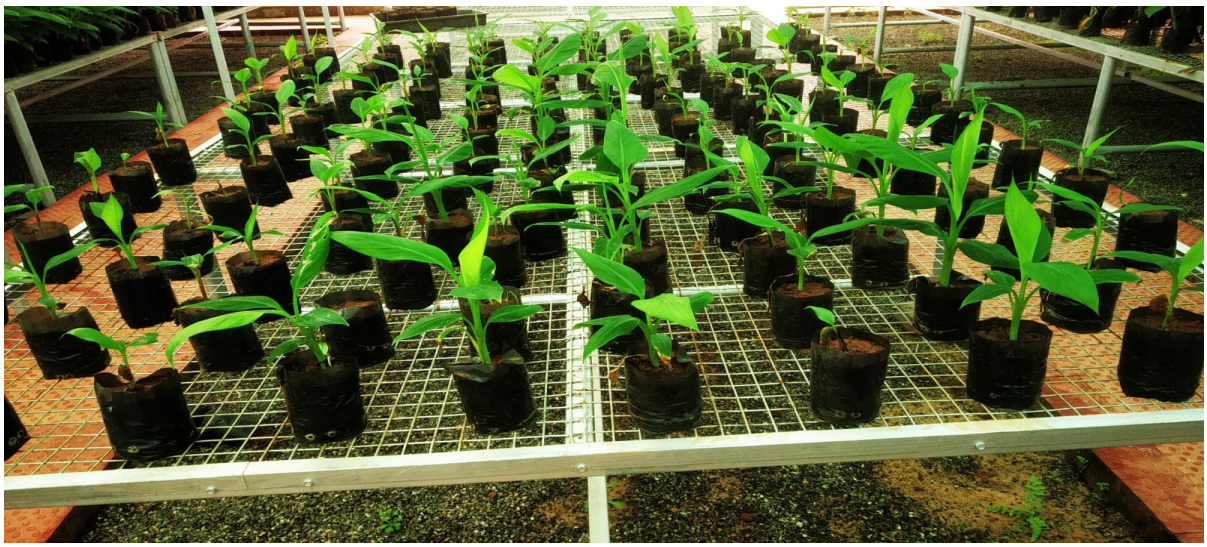


Fig. 2. Among 8 treatments, treatment 4 show average maximum no of pro-embryo/ explants (14.5±1.04)

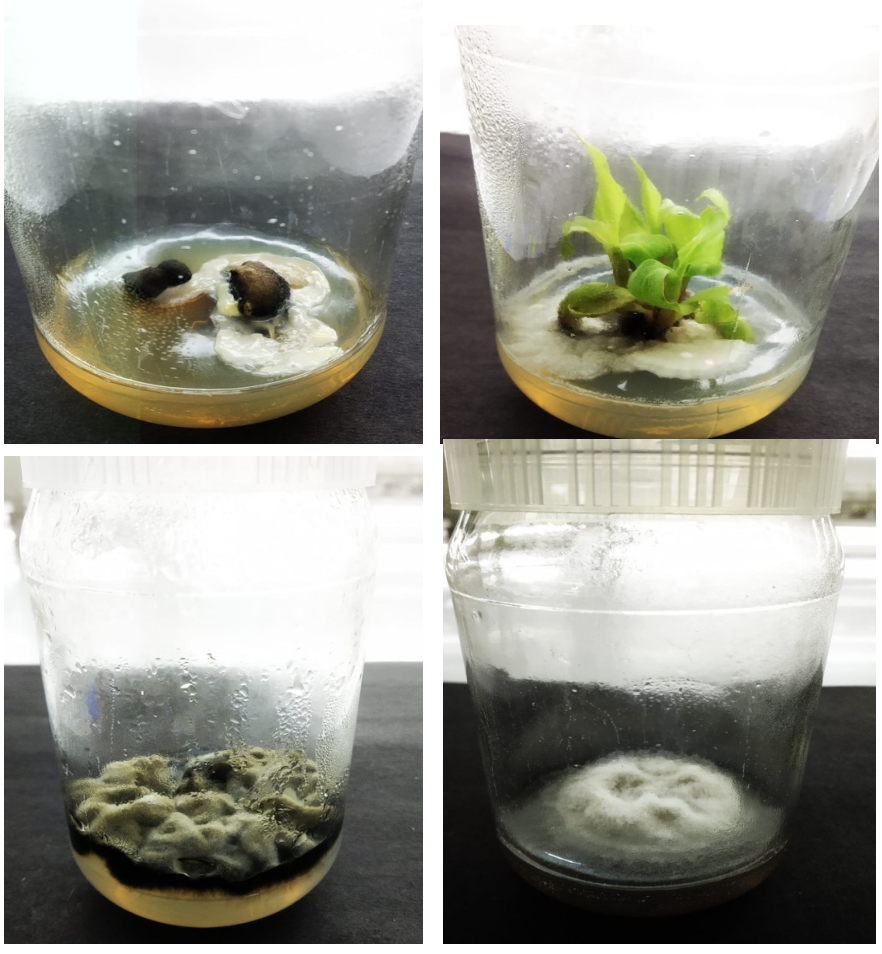
The experiment provides the results of in vitro studies on the effects of different plant growth regulators (PGRs) concentrations on root and shoot development in banana plantlets. The number of roots and the mean value are given for different PGRs and their concentrations. It can be observed that the highest number of roots [7.00] was obtained with IAA + NAA + ADS. On the other hand, the lowest number of roots was obtained NAA. The root length [20.4 cm] was obtained and the mean value for different PGRs and their concentrations, the highest root length [3.4 cm] was obtained with IAA+IBA +ADS 20 mg/l +AC 100mg/l. the lowest root length was obtained with NAA 3.0 mg/l. Finally, the plant height and the mean value for different hormones with various concentrations. The tallest plant [22.6 cm] was obtained with IAA + IBA + ADS 20mg/l + AC 100 mg/l, while the shortest plant [6.9 cm] was obtained with NAA 0.5 mg/l. the result suggests that the combination of IAA, IBA, ADS and AC at specific concentrations may be most effective for promoting root and shoot development in banana plantlets *in vitro*. However, further studies are needed to confirm these findings and to optimize the concentrations and combinations of plant hormones for successful micro-propagation of banana plants.





Different contamination recorded during plant tissue culture

Various types of contamination have been observed during plant tissue culture, including bacterial contamination, fungal contamination, viral contamination, yeast contamination and contamination by viroids. These contaminants can lead to discoloration, abnormal growth and death of the culture. Endophytic contamination, which can't be removed by surface sterilization, is also a major problem in tissue culture. Early detection of contamination is crucial to maintaining a pure culture. To eliminate contamination, appropriate antibiotics can be added to the culture medium and proper surface sterilization of explants is essential. Contamination is a frequent problem in *in vitro* culture and endophytic contamination can't be eliminated by surface sterilization. It continues to be a major issue for many plant species, as endophytic microbes and pathogens resistant to surface sterilization cause serious problems for the cultures. A broad variety of microbes, including mites, filamentous fungi, yeasts, bacteria, viruses and viroids have been found to be contaminants in plant tissue cultures. It's often not visible during the early stages of culture, but some internal contaminants emerge only during the later subculture phases and are challenging to remove. Early detection is essential for identifying microbial-free cultures. Antibiotics are used to eliminate and inhibit contamination. Cultural contamination is eradicated by adding an appropriate amount of antibiotics. Microorganisms commonly inhibit the surfaces of plants and fungi contamination can appear in culture bottles as black with creamy margins, cottony, hairy cotton or creamy white fungus. Some fungi may only appear in the culture media after several days. Contamination may also occur due to inappropriate surface sterilization of explants after inoculation and control can be achieved through proper surface sterilization using surface sterilizing agents and antibiotics. A view of various microbial contamination of in vitro cultures of red banana inoculant.



5. RESEARCH PROJECTS (LIST)

EXTERNAL FUNDED PROJECTS (2022-23)			
Sl. No	**Title**	**PI**	**Funding Agency**
1	Harnessing the potential of endophytes of Piper longum as an alternate source for piperine production: optimization of protocol for laboratory production and exploration of its anticancer properties	Dr. Nibha Gupta Principal Scientist	NMPB, GOI
2	Establishment of mass propagation and breeding facility for orchids	Dr. N. R. Nayak Senior Scientist	RKVY, GOI
3	Omics'- approach to regulate ripening and enhance fruit shelf life in banana: an important fruit crop for food security.	Dr. G.K.Surabhi Senior Scientist	RKVY, GOI

STATE PLAN FUNDED PROJECTS (2022-23)		
Sl. No	**Title**	**PI**
1	Morpho-taxonomic characterization and documentation of fungi of Odisha	Dr. Nibha Gupta Principal Scientist
2	Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from Colletotrichum sp.	Dr. Nibha Gupta Principal Scientist
3	Bioprocess optimization for enhanced recovery of Glutaminase free L-asparaginase of fungal origin	Dr. Nibha Gupta Principal Scientist
5	Standardization of efficient tissue culture based propagation methods for *Pomatocalpa Decipiens*(Lindl.) Sm. and *Phaiustankervilleae* : Rare Orchids of Odisha	Dr. N. R. Nayak Senior Scientist
6	Developing efficient micro-propagation methods for teak (tectona grandis L.) and red sanders(Pterocarpus santalinus L.) some impo0rtant and endangered forest trees of Odisha	Dr. G.K.Surabhi Senior Scientist
7	The protective diabetic neuropathy effect of Buchananialanzanspreng in streptozotocin induced type 2 diabetic rats.	Dr. A.K. Sahoo Senior Scientist
8	Ameliorative effect of Aporusa octandra against carbon tetrachloride-induced oxidative stress and hepato cellular injury in experimental rats.	Dr. A.K. Sahoo Senior Scientist
9	Field introduction and establishment studies of variously propagated wild edible fruits of Odisha	Dr. U.C. Basak Senior Scientist
9	Immunity boosting natural fruits: determination of Vitamin-C (Ascorbic Acid) and other antioxidant properties for selection of potent species to promote domestication.	Dr. U.C. Basak Senior Scientist
10	Development of alternative regeneration method of rare mangrove species of Xylocarpus spp. Through vegetative propagation.	Dr. U.C. Basak Senior Scientist

11	Assessment of antifungal activity of Combretum roxburghii and Termininalia arjuna solvent extracts against Aspergillus flavus.	Dr. S Bhatnagar Senior Scientist
12	Evaluation of non-viable seeds of Withania somnifera for biological activity.	Dr. S Bhatnagar Senior Scientist
13	Propagation and reintroduction of selected endangered species of Odisha	Dr. Kalidass C Senior Scientist
14	Standardization of in vitro regeneration techniques in red banana and establishment of red banana in Odisha climate condition.	Dr. Kalidass C Senior Scientist

6 | PUBLICATIONS

Research Paper

Year 2023

1. Baral S. and Basak U.C. (2023) Assessment of Essential Amino Acid Profiles Of Selected Underutilised Wild Edible Fruits Through UV-Vis Spectrophotometer. Eur. Chem. Bull.12 (special issue 10), 1772-1779.

2. Behera, S.; A. Kumari Panda, S. Kumar Behera, N. Gupta (2023). Media optimization,extraction, purification and characterization of Exopolysaccharide from Fusarium proliferatum: anovel source bioactive polysaccharide. Results in Chemistry 5 : 100923 (IF 2.21)

1. Das P. K., Mallia M, Kalidass C. (2023). *Lepidium didymium* L.: A new record (Brassicaceae) for Odisha, India. *The Journal of Indian Botanical Society*, page : 4-5, https://doi:10.5958/2455-7218.2023.00025.6

3. Devismita Dibya and Dr. Sunita Bhatnagar (2023)Comparative analysis of Clitorea ternatea blue and white varieties.World J of Pharmaceutical Research. 12(4), 1967-1972.

4. Gupta N and Basak UC (2023). Plant Growth Promoting Microbes and Their Possible Roles in Growth and Development of Mangroves.J Marine Biol Environ Sci 5(1): 121

5. Gupta N, Basak UC, Mohapatra K, Sandeepta S and Nayak SS (2023). Fungal Diversity in Subernarekha Mangrove Ecosystem of Odisha, India. J Marine Biol Environ Sci 5(1): 123

6. Mohapatra M. and Basak U.C. (2023) Ethno-Medicinal and Therapeutic Applications of Natural Anthraquinones: Recent Trends and Advancements. Indian Journal of Pharmaceutical Sciences. 85 (3): 544-554

7. Mohapatra Subhashree and Sunita Bhatnagar (2023) Screening of antifungal activity of *Terminalia arjuna* leaf extracts against *Aspergillus flavus.* World J of pharmacy and Pharmaceutical Sciences. 12(6), 1522-1529.

8. Panda Jiban Jyoti, P. Vetrivelkalai, B. Bhagawati, Nibha Gupta (2023). Preliminary profiling of extracellular enzymes, polysaccharide and bioactive secondary metabolites of Pseudomonas sp. and Lysinibacillus sp. isolated from Banana cultivars of Assam. Journal of Bioresearch 2 (2 : 19-26

9. Panda, J., Vetrivelkalai, P., Bhagawati, B., Gupta N. (2023). Diversity of Endophytic Fungi in Banana Cultivars of Assam India. Journal of Botanical Research. 5(2): 49-58

10. Pradhan Barsa & Sunita Bhatnagar (2023) Antioxidant, cytotoxic and phytochemical analysisOf medicinal plant *Ficus microphylla.* World Journal of Pharmaceutical Research. 12(14), 1240-125.

11. Priyadarsini Namrata and Sunita Bhatnagar (2023) Exploration of cytotoxic and antioxidant potential of

Plumbago capensis. World J of pharmacy and Pharmaceutical Sciences. 12(2), 1424-1434.

12. Rajoriya A., S.S. Tripathy and Nibha Gupta (2023). "Proximate composition, amino acids, fatty acids and mineral content of unexplored wild edible mushrooms of Odisha" Mushroom Research-An International Journal of Mushroom Research 32 (1) : 13-26.

13. Rout Pragynasini Subhashree Mohapatra and Sunita Bhatnagar(2023) Assessment of antifungal activity of *Combretum roxburghii* solvent leaf extracts against *Aspergillus flavus*. World Journal of Pharmaceutical and Life Science. 9(8), 73-76.

14. Sahoo Smruti Rekha and Sunita Bhatnagar (2023) Assessment of biological potential of non viable seeds of *Withania somnifera*(l)dunal. World J of Pharmaceutical Research. 12(7), 643-654.

15. Swain Amit Kumar and Sunita Bhatnagar (2023). Exploration of cytotoxic and antioxidant potential of *Pongamia pinnata (Family: fabaceae)*. World J of Pharmaceutical and Life Sciences. 9(1), 74-78.

Year 2022

16. Das Rashmi & Sunita Bhatnagar (2022). Evaluation of *Nyctanthes arbortristis* for antifungal activity using *Aspergillus flavus and aspergillus niger* as target experimental model. World Journal of Pharmaceutical and life sciences.8(7)127-131.

17. Khodiar Hena & Sunita Bhatnagar (2022) Phytochemical and antioxidant activity of medicinal plant *Melia azedarach*. World Journal of Pharmeceutical res.11(12),1849-1855.

18. Mallia M. and Kalidass C. (2022). *Physalis lagascae*, an invasive species found in Odisha, India. *Plantasia*, 29, In: Zoo's Print 37 (12): 11-13.

19. Mallia M. and Kalidass C. Panda, P.C. (2022) *Physalis angulata* L. var. pendula (Rydb.) Waterf. (Solanaceae): A new record for Odisha, India. *Indian Journal of Forestry*, 43 (1): 43-45.

20. Mishra Atmaja Elina, Rupa Acharya and Nibha Gupta (2022). Impact of bio inoculation, NPK and organic manure supplementation growth and development of *Dalbergia latifolia* in nursery conditions. International Research Journal of Biological Sciences.Vol.11(4):1-6.

21. Mohapatra Subhashree and Sunita Bhatnagar (2022). Phytochemical screening, antioxidant and cytotoxic activity of *Clerodendrum siphonanthus*. World Journal of Pharmaceutical and life sciences.8(3)117-122.

22. Panda Pranati and Sunita Bhatnagar (2022). Comparitive analysis of leaves and flower extracts of *Plumbago capensis*. World J pharmaceutical res. 11(16). 1462-1466.

23. Pradhan Usha Rani, Sumit Sarangi and Sunita Bhatnagar (2022). Biological evaluation of crude alkaloid extracts of *Nyctanthes arbortristis* and *Aristolochia indica*. World Journal of Pharmeceutical res.11(5), 1773-1779.

24. Rout Pragnyasini & Sunita Bhatnagar (2022). Evaluation of antifungal activity of solvent extracts of *Vallaris solanacea* against *Aspergillus flavus*. World Journal of Pharmeceutical res.11(11), 1085-1093.

25. Sahoo Jayashree & Sunita Bhatnagar (2022). Assessment of leaf extracts of *Artemisia nilagirica* against antioxidant, cytotoxic and phytochemical assays.World J of Pharmeceutical Sci. 8(10), 112-116.

26. Sahoo Smrutirekha, Pragnya Priyadarshini and Sunita Bhatnagar (2022). Assessment of antifungal activity of *Vernonia anthelmintica* solvent extracts against *Aspergillus flavus*. World Journal of Pharmeceutical res.11(6), 1028-1036.

27. Swain, S.K., Dash, U.C., Sahoo, A.K., 2022. *Hydrolea zeylanica* improves cognitive impairment in high-fat diet fed-streptozotocin-induced diabetic encephalopathy in rats via regulating oxidative stress, neuroinflammation, and neurotransmission in brain. Heliyon, 8(11).

Book

Gupta N., C. Manoharachary, S. K.Singh, Resmita Behera, P. Mekro (2023),. Fungi of Odisha : Part II . Published by Regional Plant Resource Centre, Bhubaneswar pp. 1-253.

Basak U. C. , Kalidass C. , Nayak N R. , Mekro P. (2023). Initiatives on Re-introduction of some RET and Special Group of Plants. Published by Regional Plant Resource Centre, Bhubaneswar pp. 1-154.

Research Report

Basak U.C and Mekro P. (2023). Research and Activity Report 2021-22. Published by Regional Plant Resource Centre, Bhubaneswar. pp.1-104.

7 | TRAINING & EDUCATION

Regional Plant Resource Centre provides short term training to the students of M.Sc. / B.Tech. / M/Tech. and other courses every year from January to June for a duration of 6 months. Training is imparted on various subjects pertaining to the "Advance Plant Biotechnology". The students have to submit their CV along with the forwarding letter from their Institutes head or the Project head. The applications are to be received by end of November every year. Students enroll themselves by end of December and the training starts from January (6 months). Students are allotted to various Scientists and have to work under them for completing their Thesis. Training/course completion Certificate is issued at the end of the scheduled course period infavour of the student trainee.

Detailed account of the Training provided in the Year 2022

Sl. no.	Name of the student	Topic	Supervisor/Guide
1	Subhasish Balabantaray	Studies on the propagation of three terrestrial orchids through tissue culture technique	Dr. N. R. Nayak, Sr. Scientist
2	Sankalp Subhasis Mohapatra	In Vitro Propagation Of Bulbophyllum Crassipes Hook.F: A Rare Orchid Of Odisha	Dr. N. R. Nayak, Sr. Scientist
3	Ms. Barsa Pradhan. Utkal University, BBSR.	Antioxidant, cytotoxic and phytochemical activity of medicinal plant *ficus microphylla.*	Dr. S. Bhatnagar Sr. Scientist
4	Ms. Monalisha Das, TACT, BBSR	The effect of cytokinins on shoot multiplication and elongation of cultivated banana through tissue culture.	Dr. Kalidass C Scientist
5	Ms. Sweta Suvadarsini Dikshit, SOA, BBSR	The effect of auxin hormone on root growth in cultivated bananas through tissue culture	Dr. Kalidass C Scientist

Ph.D. Pursuing/Submitted/Awarded

Sl. No.	Name of the Supervisor/ candidate	Title of the Doctoral program	University registered/Year	Status
1	**Dr. Nibha Gupta, Pr. Scientist**			
	Ms. Smitha Behera	Optimization of cultural and nutritional conditions for enhanced production of exopolysaccharide by some fungi.	Utkal University, Bhubaneswar (Reg. No.07 –Life Science 2015-16)	Submitted
	AtmajaElina Mishra	Process optimization for enhanced recovery of bioactive secondary metabolite from endophytic fungi	KIIT University,BBSR in Biotechnology , Registration no. – 21467381857	Pursuing
	Rupa Acharya	Optimization, production, purification and characterization of L-asparaginase enzyme form some fungi.	KIIT University,BBSR in Biotechnology Registration no. – 20667579426	Pursuing
	Debjani Samantray	Bioprospecting endopytic fungi from Terminalia species for antifungal properties"	Utkal University, Bhubaneswar Registration no. 001-Life Science,2020-2021	Pursuing
2	**Dr. Nihar Ranjan Nayak, Sr. Scientist**			
	Mrs. Johnnita Tirkey	Development of molecular tools for dendrobium species and their hybrids (Orchidaceae) for application in horticulture industry.	Dept. of Botany Utkal University. (Reg. No 06-Biotechnology, 2017-18)	Pursuing
	Mr. Debabrata Dash	Optimization of Various Parameters for the Production of Second Generation Bioethanol from the Efficient Biomass Producing Plants of Odisha.	Utkal University, Bhubaneswar. (Reg. No 07-Biotechnology, 2017-18)	Pursuing

3	**Dr. Giridara Kumar Surabhi, Sr. Scientist**			
	Mr. Subhankar Mohanty	A proteome approach to investigate fruit ripening and identification of key ripening proteins/genes in banana.	Utkal University, Bhubaneswar. 06-Biotechnology, 2017-18	Pursuing
	Mr. Dinesh Pradhan	Transcrption profiling and molecular characterization of candidate fruit ripening associated genes in banana.	Utkal University, Bhubaneswar. 03-Biotechnology, 2018-19	Pursuing
4	**Dr. A.K. Sahoo, Sr. Scientist**			
	Mr. Satish Kanhar	Phytochemical and Biological Evaluation of three Indian *Homalium* species with special reference to Hepatoprotective activity in CCl_4-Inuced oxidative stress in Wistar rats.	Utkal University, Bhubaneswar. (Regd. No. 02-Pharmacy-2016-17).	Pursuing
	Mr. Umesh Chandra Dash	Pharmacological profiling of *Geophilarepens* and Bacopa floribunda and evaluation of their therapeuticpotential against Alzheimer disease.	Utkal University , Bhubaneswar. (Regd. No. 10-Biotechnology-2016-17).	Awarded
	Mr. Sandeep Kumar Swain	Ethnopharmacological significance and therapeuticevaluations of *Hydroleazeylanica* in experimentallyinduced type2 diabetes in rats.	Utkal University, Bhubaneswar. (Regn.no. 01-Biotechnology-2017-18	Submitted
	Mrs. Deeptimayee Rout	Ameliorative effects of *Homaliumzeylanicum* ondiabetes induced oxidative stress and inflammation inWistar rats.	Utkal University, Bhubaneswar. (Regd. No. 11-Biotech-2016-2017).	Pursuing
5	**Dr. U. C. Basak, Sr. Scientist**			
	Ms. Swadha Baral	Essential amino acid profiling of some wild edible fruits of Odisha. (Reg. No. 04-Biotechnology-2017-18)	Utkal University, Bhubaneswar. (Reg. No. 04-Biotechnology-2017-18)	Pre-Submitted

	Ms. Abhipsa Anjeela	Identification and Characterization of some immune-enhancer bioactive compounds in wild edible fruits for therapeutic value.	KIIT University, Bhubaneswar, in Biotechnology (Regd. No. 21467481858, 2021-2022)	Pursuing
6	**Dr. C. Kalidass, Scientist**			
	Ms. Madhusmita Mallia	Study of diversity, distribution and systematics of the family Solanaceae in Eastern Ghats of India	Utkal University(Reg. No. 09-Botany-2018-19)	Pursuing
	Mrs. Sasmita Pati	Study of the ethnobotany and traditional conservation practices of the Saora tribes of Odisha	Utkal University (Reg. No. 09-Botany-2020-21)	Pursuing

8 | LIBRARY

The library of the centre has collection of books on the thrust areas of Taxonomy, Biotechnology, Medicinal and Aromatic Plants, Tissue Culture, Microbiology, Physiology and Biochemistry, Forestry and Ecology, Molecular Biology, Horticulture and Floriculture, Ornamental Plants, Orchids and many other areas. Number of periodicals and journals of leading institutions and firms on related areas of importance are subscribed by the library. Several Indian Journals of repute are included as annual subscription.

9 | HERBARIUM

The Centre has a modern Herbarium with a collection of 14,000 accessions belonging to 1600 species. The herbarium specimens have been digitized and made available to researchers as well as scientific communities through a web-based application.

10 | EX-SITU CONSERVATION & GERMPLASM COLLECTION

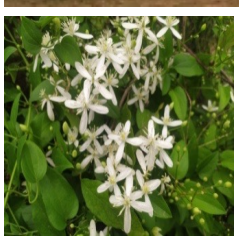
RPRC has its rich living collections of different plant groups like cacti and other succulents, wild and exotic orchids, species with fragrant flowers, endangered and threatened plants, medicinal plants, mangroves, palms, bamboos, wild edible fruit plants, cultivars of *Hibiscus* and Roses. These have been introduced to the living collection division and are being studied.

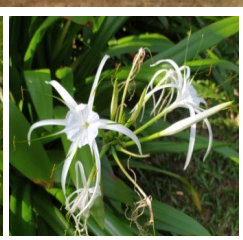
Wild Edible Fruits Garden : In order to create awareness among various stake holders including foresters, plant lovers, researchers and general citizen about various wild edible fruits occurring in Odisha, RPRC has created an *ex situ* conservation garden housing more than 110 species of fruits, nuts & berries. Since plant conservation and research are major objectives of the centre, this germplasm collection is also meant to provide important wild fruit bioresource for undertaking research on propagation, cultivation, analysis and utilization.

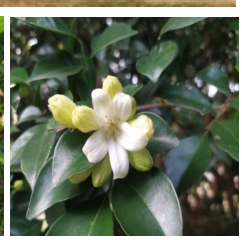
RET Corner: Conserved more than 35 RET species like *Lasiococca comberi, Hildegardia populifolia, Cycas sphaerica, Homalium tomentosum, Hypericum gaitii, Cordia maclleodii, Gnetum ula, Heritiera fomes, Heritiera littoralis, Homalium tomentosum etc.*

Jagannath Vatika: A special garden housing 125 species of plants used in different rituals of Lord Jagannath and the plants are grouped under 7 categorized such as -1.Construction of chariots and special carts to transport the holy logs of Neem for making the idols.2.Selection of holy Neem plants for making the idols and special characteristics of the site.3.Making a special floral crown "Tahia" for the deities.4.Preparation of "Dasamula"- an Ayurvedic drug.5.Preparation of fragrant herbal oil called "Phuluri"6. Leaves and flowers used in different attires, rituals and festivals and 7.Fruits and seeds used in daily rituals, attires and special occasions.

Fragrance Flower Garden: The iconic Botanic Garden (Ekamrakanan) of RPRC offers visitors a living museum of native and exotic plant collections of fragrant flowers, both wild and cultivated, in its themed section called 'Fragrant Flower Garden'. With a collection of more than 70 species of plants with scented flowers, the fragrance garden established in a unique landscape over about 3.5 acres of land and thus a must-visit site for everyone. The centrally placed giant Ashoka Chakra is surrounded by several landscapes represented by majority of Magnolias, Jasmines, Gardenias, Tabernae montanas and many more scented flower groups inter-connected with network of visitors path and shed to enjoy the beauty and fragrance of the of the garden.







Rose Garden: The Centre has collection of around 1000 varieties of roses. The available varieties include Alec's Red, Black Lady, Double delight, French perfume, Nurjahan, Surkhab, Tiara etc,

Palmetum: Representing nearly 60 species of palms including *Archontophoenix alexandrae, Calamus spp, Corypha umbraculifera, Dypsis lutescens, Livistonia chinensis, Ravenea rivularis* etc.

Bambusetum : Having collection of around 30 species of bamboos like *Arundinaria chino, Arundo donax, Bambusa balcooa, Dinochloa maclellandii, Melocanna baccifera, Phyllostachys nigra, Pseudosasa japonica* etc.

Hibiscus Garden : The garden has a collection of 52 varieties of *Hibiscus* species.

Medicinal Plant Garden: This germplasm garden houses 250 species of medicinal plants collected from all over the country.

Cacti and other Succulents: The Centre houses more than 1000 Varieties and cultivars of cacti & succuents, both for sale and display for visitors.

Orchidarium: Having germplasm collection of nearly 100 species of Orchids. Some species/hybrid orchids are also displayed in orchadarium in botanical garden & many hybrid orchids are available for sale.

11 | FLOWER SHOW 2023

The Regional Plant Resource Centre (RPRC has organised the Annual Flower Show 2023 in the premises of the Botanic Garden of RPRC (Ekamra Kanan) on 14th -15th January, 2023 in association with Plant Lovers' Association (PLA), Bhubaneswar with the support of Odisha Mining Corporation Ltd., Directorate of Horticulture, Odisha and Odisha Forest Development Corporation Ltd.

Shri Pradip Kumar Amat, Hon'ble Minister, Forest, Environment & Climate Change, Panchayati Raj & DW, Information & Public Relations, Odisha has inaugurated the Annual Flower Show, 2023 as Chief Guest. Shri Amat along with Rajya Sabha MP Smt Sulata Deo visited various floral displays, exhibition stalls and floral arrangements made by Regional Plant Resource Centre, Bhubaneswar.

The Hon'ble Minister has released Research and Activity Report 2021-22 of RPRC, launched e-Commerce Portal for nursery plants of RPRC and also inaugurated Pop-up irrigation system installed in the campus.

Smt. Pusazhule Mekro, IFS, PCCF & Chief Executive, RPRC accompanied both the Chief Guest and Guest of Honour during the Inaugural Function and briefed the whole events of the Flower Show.

All Scientists of RPRC, Senior Officers and office staff of RPRC and Plant Lovers Association were also present during the Inaugural Function. An attractive "Floral Gate" has been erected at the entry point of the Flower Show premises, which has become a major attraction of the event.

A total fifteen organizations/ firm, thirty nurseries (open space), sixteen stalls have participated and exhibited ornamental plants for display and sale through Plant Bazar. Apart from Cut Flower display, a "Orchid" and Ferns Rare Varieties are on display.

As a part of the Flower Show, a "Garden Competition" event was organized in the Capital city of Bhubaneswar to encourage the residents for raising Gardens and growing plants to add to the beauty of the City. The winners of Garden Competition are being awarded with prizes and trophies in the Valedictory Function held on 15th January, 2023. Shri Debidutta Biswal, IFS, PCCF & HoFF, Odisha has graced the Valedictory Function held on 15th January, 2023 as Chief Guest. Shri Balwant Singh, IAS, Managing Director, Odisha Mining Corporation Ltd., Shri Suresh Pant, IFS, Addl. PCCF & Managing Director, Odisha Forest Development Corporation Ltd. and Shri Rohit Kumar Lenka, IFS, Director of Horticulture, Odisha had graced the occasion as the Guest of Honour.

Under the able guidance of the Chief Executive, all scientists, Officers, staff, students and workers of RPRC had put their best efforts to make this Annual Flower Show, 2023 a success. The financial assistance from OMC, Directorate of Horticulture, Odisha and OFDC Ltd. and help and assistance received from different participating institutions and individuals are gratefully acknowledged.


Inauguration of Flower Show 2023 in public






Visit to RPRC Stall & Floral Display




Floral Gate; Photopoint

RPRC Stall & Display

Thermatic Flower Display

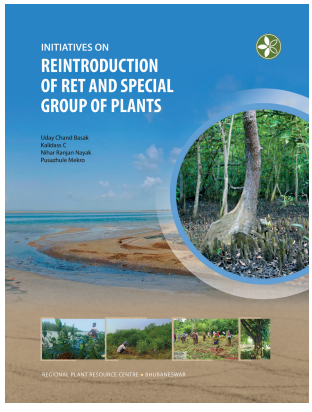
Prize & Certificate Distribution

PLA Book/ Souvenir Release

Inauguration of Automated Sprinkler Irrigation Systems in RPRC

12 | RELEASE OF BOOKS AND OTHER PUBLICATIONS

RPRC Publications (2023)Released on occasion of World Environment Day (June 5th , 2023)

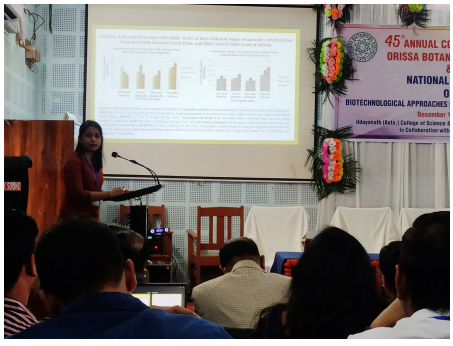


Book

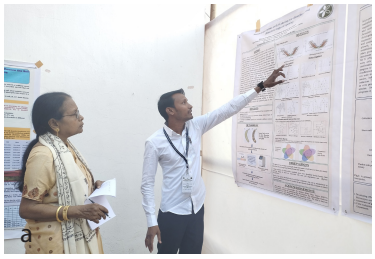
- Fungi of Odisha, Part-II
- Initiations of Reintroduction of RET and other Speial Group of Plants
- Research and Activity Report 2021-22

13 | SEMINAR/ CONFERENCES




Attended & poster presented on "Exploring Immune-boosting ascorbic acid (Vit-C) in some wild edible fruits used by tribals of Odisha" in **45th Annual Conference of Orissa Botanical Society & National Seminar on Biotechnological Approaches for Mitigating Climate Change** at U.N Autonomous College, Adaspur, Cuttack (2022).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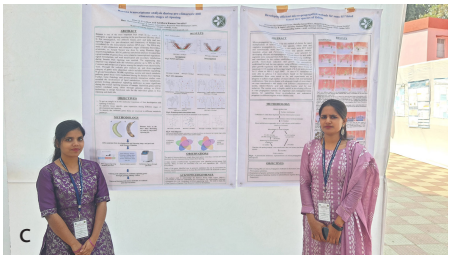

c

a. Attended & poster presented on **Banana transcriptome analysis during pre-climacteric and climacteric stages of ripening'**, **b&c**. **Developing efficient micro-propagation methods for some RET listed forest tree species of Odisha',** at 23rd Odisha Bigyan 'O' Paribesh Congress &, National Seminar on Science and Technology in Combating Climate Change, Organised by Sambalpur University, & Odisha Environmental Society on dt.26-27 November, 2022.

14 | EXPOSURE VISIT OF VARIOUS INSTITUTIONS/ ORGANIZATIONS TO RPRC

Exposure visit of Students from different educational institutions (2023)

Exposure visit of Students from OUAT, Bhubaneswar (2023)

15 | BALANCE SHEET

REGIONAL PLANT RESOURCE CENTRE

NAYAPALLI, BHUBANESWAR-751015

BALANCESHEET AS ON 31.03.2023

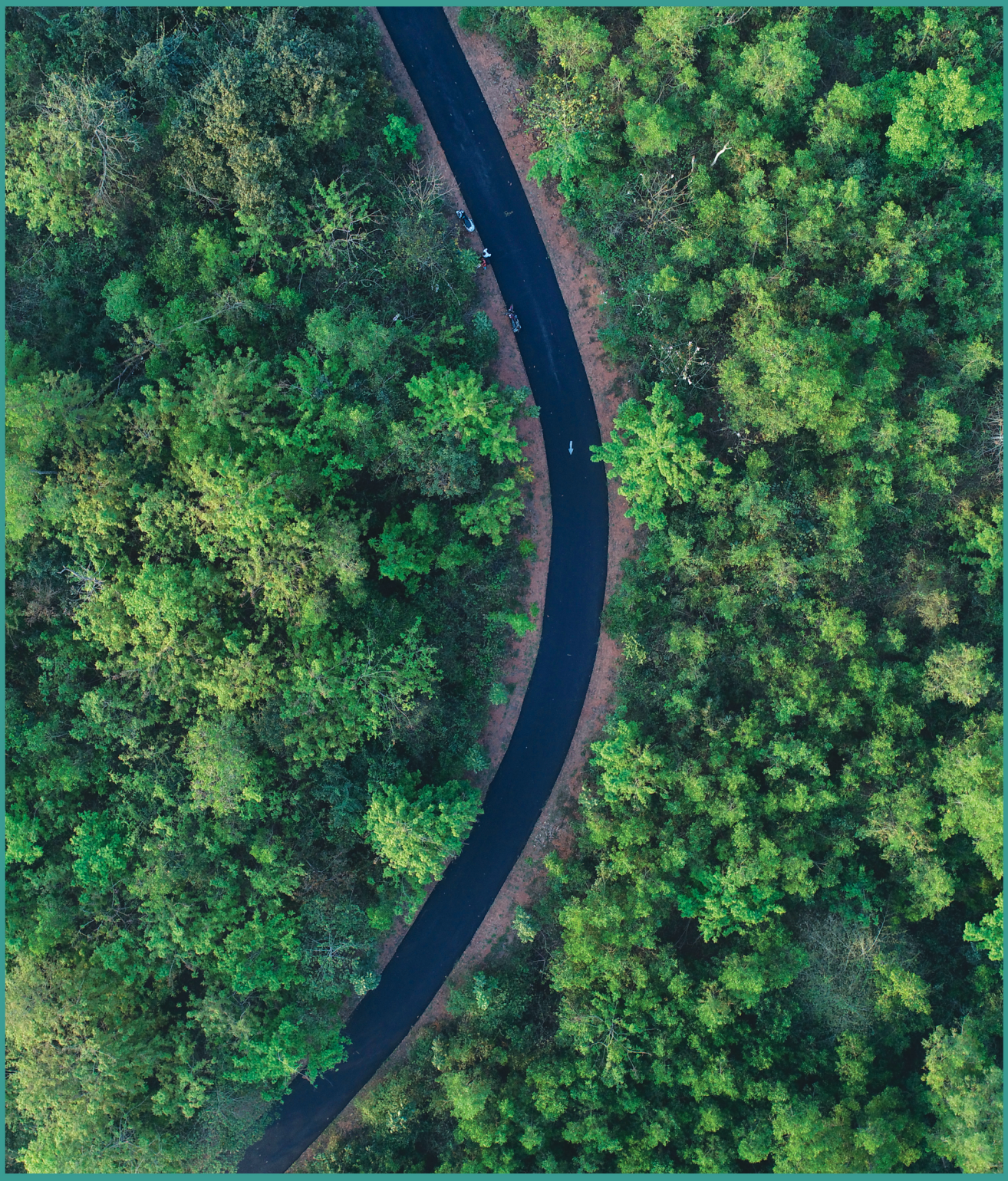
LIABILITIES	SCHEDULE	AMOUNT(RS.)	ASSETS	SCHEDULE	AMOUNT(RS.)
General Fund	1	8,49,10,729	Fixed Assets	5	8,26,06,422
Grant for Non-recurring Expenses	2	15,25,75,414	Work-in-Progress	6	3,69,86,813
Advance Received for Contract Work	3	72,20,370	Fund Transfer to opening new scheme account		45,65,034
Current Liabilities	4	1,05,19,807	Current Assets		
			Loans & Advances	7	10,16,05,799
			Cash in Hand		3,210
			Cash at Bank		2,94,59,042
Total		25,52,26,320	Total		25,52,26,320

For PARTHA S MISHRA & CO.
Chartered Accountants
Sanjaya Kumar Patra
CA S.K. Patra (FCA, DISA)
Partner, M. No- 301929

PARTHA S MISHRA & CO. FRN 324...E BBSR Chartered Accountants

Administrative cum Finance Officer
Regional Plant Resource Centre
Bhubaneswar

UDIN: 23301929BGWNHR1286
Place: Bhubaneswar
DATE: 27/09/2023



Regional Plant Resource Centre
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