Tissue culture, omics analysis and bioactivity studies of Alpinia spp.

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Submitted By : Dr. Binod Kumar Mahto

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PROPOSAL DETAILS

(SRG/2022/001383)

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Technical Details :

Scheme :	Start-up Research Grant		
Research Area :	Plant Sciences (Life Sciences)		
Duration :	24 Months	**Contact No :**	+918010140158
Date of Birth :	30-Jan-1986		
Nationality :	INDIAN	**Total Cost (INR) :**	32,66,916

Project Summary :

Alpinia genus have some of the widely used medicinally relevant and edible species. The genomic, transcriptomic and metabolomic complexities of some Alpinia spp. make it difficult to decipher the important metabolic pathways and their regulation. We aim to analyze the transcriptome and metabolome of two important species, A. galanga and A. calcarata. Unraveling the underlying regulatory mechanisms of specialized metabolic pathways would increase the amenability of these Alpinia spp. towards genetic engineering protocols, tissue culture as well as their bioactive studies. Further, rapid regeneration through tissue culture protocol and bioactivity studies would be developed and performed, respectively to increase their commercial and pharmaceutical gains.

Objectives :

- Standardization of an in vitro multiplication protocol for Alpinia calcarata and Alpinia galanga plants.
- Isolation and quantification of secondary metabolites (flavonoid).
- Comparative Transcriptome analysis in different plant tissues of A. calcarata and A. galanga plants.
- In vitro antifungal activity against fungal pathogen Colletotrichum spp. by using rhizome extract.

Keywords :

Alpinia galanga, A. calcarata, Tissue Culture, Transcriptome, Bioactivity

Expected Output and Outcome of the proposal :

Alpinia spp. are highly economically important medicinal plant. Till date, tissue culture studies of regeneration and organogenesis are reported very few in Alpinia spp. such as A. calcarata and A. galanga. In the current study, we aim to analyze the transcriptome and metabolome of two important species, A. galanga and A. calcarata. Unraveling the underlying regulatory mechanisms of specialized metabolic pathways would increase the amenability of these Alpinia spp. towards genetic engineering protocols, tissue culture as well as their bioactive studies. Further, rapid regeneration through tissue culture protocol and bioactivity studies would be developed and performed, respectively to increase their commercial and pharmaceutical gains. Simultaneously, we will evaluate in vitro antifungal and antibacterial potential of rhizome extracts against a spectrum of plant pathogens, as well as if feasible human pathogens too. Concurrently, we will be the establishment of industrial collaboration to translate our product. Also, we will transfer knowledge to local farmers to grow our regenerated plants in the field for production in large quantities. Participation in world-leading conferences, publication in high-impact journals.

Technical Details

Title: Tissue culture, omics and bioactivity studies on rhizomes of *Alpinia* spp.

1. State of the art

Alpinia sp. are listed as important medicinal and edible perennial herbs belonging to the Zingiberaceae family, which contains approximately 250 species (Ma et al. 2017; http://www.theplantlist.org; https://www.ipni.org). Among them, *A. galanga* and *A. calcarata* are domesticated and widely cultivated in the tropical region of Southeast Asian countries for culinary purposes. Their rhizomes are used in popular folk medicines to treat illnesses such as indigestion, gastralgia, vomiting, enterozoa, rheumatism, bronchitis and asthma (Tushar et al., 2010; Singh et al. 2020; Zhang et al. 2020). However, the productivity of *A. calcarata* and *A. galanga* experiences a shortfall through conventional agricultural practices which are not sufficient to meet the demand. Moreover, the conventional breeding method is also very difficult in such rhizomatic plants due to lack of seed setting processes. The rhizomes are reported to possess numerous bioactivities such as antifungal, antibacterial, antiulcer, anti-oxidant, anti-viral, antiemetic, antibacterial, cardiovascular, hypoglycemic, antitumor, cardio-protection, antianxiety and neuroprotection activities (Ma et al. 2016; Zhang et al. 2020). Studies on rhizome extracts suggested their potential to be used as antibacterial and antifungal agents (Prasad et al. 2016; Joseph et al. 2012). Phytochemical screening of *A. calcarata* and *A. galanga* rhizomes have revealed the dominance of secondary metabolites that majorly belong to the class of flavonoids, steroid glycosides and alkaloids (Rahman and Islam 2015). However, the molecular and biosynthetic machinery underlying some unique secondary metabolites in these rhizomatic plants remains unexplored. Also, these plants are affected by various diseases such as rhizome rot and bacterial wilt (Babu et al. 2016); hence vegetative propagation of these plants seems difficult to produce disease-free plants. Tissue culture studies of regeneration and organogenesis are reported in several plants in Zingiberaceae family, but few are directed towards *A. calcarata* and *A. galanga*, which make them insufficient to replicate the propagation on a large-scale. Therefore, during present investigation, we aim to evaluate and optimize the protocol for *invitro* multiplication of *A. calcarata* and *A. galanga* for large-scale production. For fundamental understanding, we will also analyse the transcriptome and metabolome and subsequently focus on the molecular mechanism behind high-abundance secondary metabolites in these plants. Simultaneously, we will evaluate *in vitro* antifungal and

antibacterial potential of rhizome extracts against a spectrum of plant pathogens, as well as if feasible human pathogens too.

2. Origin of the Proposal:

A. calcarata and *A. galanga* are one of the important medicinal plants and it belongs to the Zingiberaceae family. These plants are rhizomatous perennial herbs, used in the traditional herbal medicine for the treatment of many diseases or illness such as cancer, indigestion, gastralgia, fever, ulcers, whooping cough, rheumatism, vomiting, enterozoa, rheumatism, bronchitis, sexually transmitted diseases and asthma (Abubakar et al. 2018; Rao et al. 2011; Ma et al. 2016; Tushar et al., 2010; Singh et al. 2020; Zhang et al. 2020). *A. calcarata* and *A. galanga* are mainly cultivated in the tropical region of Asia including India, China, Sri Lanka, Malaysia and Indonesia (Singh et al. 2020). Over the years, many studies revealed that pharmacological and phytochemical potency on the *Alpinia* species such as *A. oxyphylla, A. calcarata, A. galanga, A. zerumbet* and *A. officinarum* (Merh et al. 1986; Sun et al. 2016; Li et al. 2013; Niu et al. 2020). Phytochemical screening of extract from rhizome and leaves showed the presence of metabolites like flavonoids, tannins, alkaloids, polyphenols and steroid glycosides (Rahman and Islam 2015). Among these terpenoids and flavonoids are the major active ingredients of *Alpinia* sp. and successfully reported various pharmacological activities (He et al. 2019). However, to date, the study of the molecular mechanism and biosynthesis pathway of flavonoids has not been reported. The aqueous and ethanolic extracts from rhizomes showed antifungal, antibacterial, antioxidant, anthelmintic, antidiabetic and aphrodisiac effects (Arambewela et al. 2004; Arambewela et al. 2005; Arambewela et al. 2009; Raj et al. 2011, Kaladhar and Narasinga 2014; Rahman and Islam 2015). The essential oil extracted from roots and rhizome of *Alpinia galanga* and *A. calcarata* showed antifungal activity against the various fungal pathogens causing devastating disease in plants such as *Rhizoctonia solani, Aspergillus aculeatus, A. awomori, A. niger, Fusarium oxysporum and Candida albicans* (Handajani and Purwoko 2008; Arambewala et al. 2010; Joseph et al. 2012; Prasad et al. 2016).

According to a recent World Bank report; “Medicinal Plants: Rescuing a global heritage”, a large number of medicinal plants are being over-harvested and could soon become extinct, unless, strong conservation measures are introduced by developing countries. Cultivation of valuable species in experimental conditions is one of the approaches and in this context, the method of multiplication of plants by biotechnological methods has come as a

boon that produces an enormous number of identical plants. In vitro multiplication provides many advantages over conventional methods of propagation and is a helpful aid for rapid clonal propagation of superior genotypes having desirable traits. *A.calcarata* and *A. galanga* are affected by several diseases such as rhizome rot and bacterial wilt (Babu et al. 2016); hence vegetative propagation of these plants is very difficult to produce disease free plants. In vitro multiplication studies of regeneration and organogenesis has been reported in many species of Zingiberaceae family, but in the case of *A. calcarata* and *A. galanga* only a few has been reported which is not sufficient for large scale multiplication. Borthakur et al. (1998) reported a micropropagation protocol of a medicinally important herb *A. galanga* by using emerging buds of rhizome and the rate of multiplication was not efficient. Bhowmick et al. (2016) demonstrated the propagation study of *A. calcarata* for their genetic stability by using the RAPD and ISSR molecular markers. Therefore, to increase the productivity of medicinal plants at a large scale, we have to develop a highly efficient protocol for the *A. calcarata* and *A. galanga* plants.

So, in the current proposal, we have to develop a highly efficient *in vitro* multiplication protocol for *A. calcarata* and *A. galanga* plants. Also aimed for transcriptome study to identify the biosynthesis pathway of flavonoids and their molecular mechanism, quantitative analysis of chemical constituents like terpenoids and flavonoids, and in vitro antifungal activity against fungal pathogen *Colletotrichum* sp. causing devastating disease in many cultivated vegetable crops such as chili, tomato, pea etc.

3. Research Plan

3.1 Methodology:

For the *in vitro* multiplication, we have to develop a highly efficient regeneration protocol for *A. calcarata* and *A. galanga* by using rhizome bud as an explant. For the regeneration, we have to optimize with the various type of phytohormones combinations and concentration particularly of cytokinins and auxins in plant growth medium, and the culture conditions light and temperature. To determination of flavonoids contents, sample will be prepared from leaves and rhizome of regenerated and wild-type plants of *A. calcarata* and *A. galanga* using methanol extract methods, and analyze by using high-performance liquid chromatographic technique (Tao et al. 2006). Comparative transcriptome study of *A. calcarata and A. galanga* through high-throughput RNAseq method. For the RNA sequencing, total RNA will be extracted form different plant tissues such as root, rhizome and leaf of *A. calcarata and A. galanga*. Extraction

of essential oils from these plants and *in vitro* antifungal activity will be conducted against fungal pathogen *Colletotrichum* sp., which causing devastating disease in many cultivated vegetable crops such as chili, pea and tomato etc.

3.2 Delivery and milestones:

The deliveries and milestones of this 2-years project, as summarized in the Gannt chart below, alongside the objectives/activities and task breakdown. Simultaneously, we will be establishment of industrial collaboration to translate our product. Also, participation in world-leading conferences, publication in high impact journals and application for grants to expand my group activities.

Table 1: Gantt chart with the objectives/activities as well as the milestones.

Objectives/ activities	**Year 1**				**Year 2**			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Objective 1: Standardization of an *in vitro* multiplication protocol for *Alpinia calcarata* and *A. galanga*								
Objective 2: Isolation and quantification of secondary metabolites (flavonoid)								
Objective 3: Comparative Transcriptome analysis in different plant tissues of *A. calcarata* and *A. galanga* plants								
Objective 4: *In vitro* antifungal activity against fungal pathogen *Colletotrichum* sp. by using rhizome extract								

4. Key publications of the Investigator during the last 5 years

- Sagar S Arya, **Binod Kumar Mahto**, Meenu Senger, Jim E Rookes, David M Cahill, Sangram K Lenka. **Metabolic engineering of rice cells with vanillin synthase gene (*VpVAN*) to produce vanillin (2022)**. *Molecular Biotechnology*, Springer Nature. (https://doi.org/10.1007/s12033-022-00470-8).

- Nidhi Dongre, Divyani Kumari, **Binod Kumar Mahto**, Sagar Sanjay Arya, Ashraf Saifullah, Sangram Keshari Lenka. **Mutagenomics for functional analysis of *plant***

***genome using CRISPR* library screen (2021)**. *RNA-Based Technologies for Functional Genomics in Plants*, Springer Nature, pp 339-367. (DOI: 10.1007/978-3-030-64994-4).

- **Binod kumar Mahto**, Anjulata Singh, Manish Pareek, M V Rajam, Swatismita Dhar-Ray, P M Reddy. **Host-induced gene silencing of *conidial morphology 1* gene (*CgCOM1*) to confers resistance against anthracnose in chilli and tomato (2020)**. *Plant Molecular Biology*, Springer Nature; 104:381-395. (PMID: 32803478, DOI: 10.1007/s11103-020-01046-3).

- Sagar S Arya, **Binod Kumar Mahto**, Thakku R Ramkumar, Sangram K Lenka. **Sharpening gene editing toolbox in Arabidopsis for plants (2020)**. *Journal of Plant Biochemistry and Biotechnology*, Springer nature, 29: 769-784. (DOI: 10.1007/s13562-020-00606-4).
- **Binod kumar Mahto**, Amit Katiyar, Sangram K Lenka, Kailash C Bansal. **Small RNA technology for plants abiotic stress tolerance (2020)**. *Plant small RNA: Biogenesis, Regulation and application, 1st edition*, *Acedamic press*: Elsevier, Pages 521-541. (doi.org/10.1016/B978-0-12-817112-7.00023-7).

- **Binod kumar Mahto**, Poonam Sharma, M V Rajam, P M Reddy, Swatismita Dhar-Ray. **An efficient method for *Agrobacterium*-mediated genetic transformation of chilli pepper (*Capsicum annuum* L) (2018)**. *Plant Physiology Repots* (Formerly *Indian Journal of Plant Physiology)*, Springer Nature, 23:573-581. (https://doi.org/10.1007/s40502-018-0389-1.

5. Bibliography

Borthakur M, Hazarika J, Singh R: A protocol for micropropagation of *Alpinia galanga*. Plant Cell, Tissue and Organ Culture 55: 231-233 (1998).

Ma X-N, Xie C-L, Miao Z, Yang Q, Yang X-W: An overview of chemical constituents from *Alpinia* species in the last six decades. RSC advances 7: 14114-14144 (2017).

Singh S, Sahoo BC, Kar SK, Sahoo A, Nayak S, Kar B, Sahoo S: Chemical constituents Analysis of *Alpinia galanga* and *Alpinia calcarata*. Research Journal of Pharmacy and Technology 13: 4735-4739 (2020).

Sun Z, Kong X, Zuo L, Kang J, Hou L, Zhang X: Rapid extraction and determination of 25 bioactive constituents in *Alpinia oxyphylla* using microwave extraction with ultra high performance liquid chromatography with tandem mass spectrometry. Journal of separation science 39: 603-610 (2016).

Prasad L, Rana V, Raina A: Antifungal activity of essential oils obtained from roots and rhizomes of *Kaempferia galanga* Linn., *Alpinia galanga* (Linn.) and *Alpinia calcarata* Roscoe. against *Rhizoctonia solani*. Ind Phytopathol 69: 499-500 (2016).

Niu Q, Gao Y, Liu P: Optimization of microwave-assisted extraction, antioxidant capacity, and characterization of total flavonoids from the leaves of *Alpinia oxyphylla* Miq. Preparative biochemistry & biotechnology 50: 82-90 (2020).

He B, Xu F, Yan T, Xiao F, Wu B, Wang Y, Bi K, Jia Y: Tectochrysin from *Alpinia oxyphylla* miq. Alleviates Aβ1–42 induced learning and memory impairments in mice. European journal of pharmacology 842: 365-372 (2019).

Arambewela LS, Arawwawala LD, Ratnasooriya WD. Antinociceptive activities of aqueous andetahnolic extracts of *Alpinia calcarata* rhizomes in rats. *J Ethnopharmacol.* 2004;95:311–6.

Arambewela LS, Arawwawala LD. Antioxidant activities of ethanolic and hot aqueous extracts of*Alpinia calcarata* rhizomes. *Aust J Med Herbalism.* 2005;17:91–4.

Arambewela LS, Arawwawala LD, Ratnasooriya WD. Gastroprotective activity of hot ethanolicextract of *Alpinia calcarata* rhizomes in rats. *Ceylon J Med Sci.* 2005;48:1–11.

Arambewela LS, Arawwawala LD, Ratnasooriya WD. Effect of *Alpinia calcarata* rhizomes onethanol-induced gastric ulcers in rats. *Phcog Mag.* 2009;4:226–31.

Ratnasooriya WD, Jayakody JR. Effects of aqueous extract of *Alpinia calcarata* rhizomes onreproductive competence of male rats. *Acta Biol Hung.* 2006;57:23–35.

Arambewela LS, Arawwawala LD, Ratnasooriya WD. Hypoglycemic and antihyperglycemic activitiesof the aqueous and the ethanolic extracts of *Alpinia calcarata* rhizomes in rats. *Phcog Mag.* 2009;5:412–8.

Raj N, Nadeem S, Jain S, Raj C, Nandi CK. Ameliorative effects of *Alpinia calcarata* in alloxan-induced diabetic rats. *Digest J Nanomat Biost.* 2011;6:991–7.

Arawwawala DA, Arambewela SR, Ratnasooriya DW. *Alpinia calcarata* Roscoe: A Rich Source ofPhytopharmaceuticals in Sri Lanka. *Nat Prod J.* 2012;2:263–9.

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

Equipment available	Generic Name of Equipment	Model, Make & year of purchase	Remarks including accessories available and current usage of equipment
PI & his group	Nil	-	-
PI's department (Central Instrumentation Lab)	Thermal Cycler	Eppendorf, 2019	80%
	Microcentrifuge	Eppendorf, Model No. 5418R, 2019	100%
	-80° deep freezer	Eppendorf,	100%

		U410862019	
	Orbital shaker/incubator	2019	100%
	Electronic balances	2019	
	Submarine gel electrophoresis system	Eppendorf, 2019	90%
	Spectrophotometer	Eppendorf,2019	90%
	Chemiluminescence imaging system	Eppendorf,2019	100%
	Vortex	Eppendorf, 2019	100%
	Fermentor	Eppendorf BIOFLO-120,205LPM, 2019	90%
	pH Meter		100%
Other Institute(s) in the region			

Budget Details

Institution wise Budget Breakup :

Budget Head	Ranchi University	Total
Research Personnel	8,63,040	**8,63,040**
Consumables	7,50,000	**7,50,000**
Travel	1,00,000	**1,00,000**
Equipment	8,56,884	**8,56,884**
Contingencies	1,00,000	**1,00,000**
Other cost	3,00,000	**3,00,000**
Overhead	2,96,992	**2,96,992**
Total	**32,66,916**	**32,66,916**

Institute Name : *Ranchi University*

Year Wise Budget Summary (Amount in INR) :

Budget Head	Year-1	Year-2	Total
Research Personnel	4,31,520	4,31,520	**8,63,040**
Consumables	4,50,000	3,00,000	**7,50,000**
Travel	50,000	50,000	**1,00,000**
Equipments	8,56,884	0	**8,56,884**
Contingencies	50,000	50,000	**1,00,000**
Other cost	1,00,000	2,00,000	**3,00,000**
Overhead	1,48,496	1,48,496	**2,96,992**
Grand Total	**20,86,900**	**11,80,016**	**32,66,916**

Research Personnel Budget Detail (Amount in INR) :

Designation	Year-1	Year-2	Total
Junior Research Fellow *To complete research wet lab work such as in vitro propagation, bioassay, and transcriptome analysis. We required one Junior Research Fellow.*	4,31,520	4,31,520	**8,63,040**

Consumable Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Total
To achieve the objectives we need various chemicals, plasticware, and glassware.	4,50,000	3,00,000	**7,50,000**

Travel Budget Detail (Amount in INR) :

Justification (Inland Travel)	Year-1	Year-2	Total
Local travel is needed to collect plant material, attend conferences, local meetings, project activities, workshops, and training events.	50,000	50,000	**1,00,000**

Equipment Budget Detail (Amount in INR) :

Generic Name ,Model No. , (Make)/ Justification	Quantity	Spare time	Estimated Cost
Submerine Agarose Gel electrophoresis BR Biochem 31DN Horizontal Electrophoresis Cell System (BR Biochem, India) *To check the quality of DNA and RNA as well as analysis of PCR amplified amplicon.*	1	30 %	**35,019**
Refrigrator 4 degree Blue Star (Blue Star) *To maintain the various fluids, lower chemicals, and biological activity in a controlled environment (refrigerated space), so that they are kept in good condition the lower the temperature.*	1	0 %	**25,000**
Ice Making Machine BR Biochem-40 Ice Making Machine (BR Biochem, India) *Ice flakes and cubes are very important for maintaining the conditions of proteins, enzymes, and other reagents during lab experiments, for a transit time outside the refrigerator and deep freezers. This Follet ice machine makes rapidly makes ice and keeps it unmelted for a long time. The ice pellets keep the enzyme samples protected for a transit time outside the fridge.*	1	100 %	**1,10,000**
Orbital Shaker Incubator REMI RIS 24 Plus Orbital Shaking Incubator (REMI, India) *Extraction of secondary metabolites and suspension culture.*	1	40 %	**2,00,000**
Laminar Air Flow Laminar Air flow Horizontal ÁCCO brand (ACCO, USA) *For the tissue culture study.*	1	40 %	**1,45,000**
Pipettes Rainin Mettler Toledo Pipettes (Mettler Toledo) *A tool used to dispense measured volumes of liquids.*	1	0 %	**60,615**
Minus 20 Deep Freezer Bluestar (Blue Star, India) *To maintain the various fluids, lower chemicals, and biological activity in a controlled environment (refrigerated space), so that they are kept in good condition the lower the temperature.*	1	0 %	**50,000**
Water Bath Water Bath with digital PID Controller (RIVOTEK) *For DNA and RNA isolation.*	1	0 %	**35,250**
Computer Dell (DELL) *For transcriptome analysis as well as for bioinformatics work.*	1	0 %	**1,00,000**
Power Supply BI-HE-500 (BR Biochem, India) *To run the agarose gel electrophoresis.*	1	30 %	**36,000**
WEIGHING BALANCE BRAS 224 (BR Biochem, India) *For the weighing of chemical powders.*	1	30 %	**60,000**

Contingency Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Total
As per the government rule, contingency grants will be used for the research lab like stationery, accessories, registration fees for conferences.	50,000	50,000	**1,00,000**

Overhead Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Total
Overhead expenses for the institute including infrastructural facilities and laboratory.	1,48,496	1,48,496	**2,96,992**

Other Budget Detail (Amount in INR) :

Description/Justification	Year-1	Year-2	Total
Outsourcing For the quantification of secondary metabolites, Sequencing, synthesis of primers, and Transcriptome study.	1,00,000	2,00,000	**3,00,000**

BIO-DATA

1. Name and full correspondence address: **Dr Binod Kumar Mahto**
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2. Email(s) and contact number(s): **krvinod09@gmail.com; +91-8010140158**

3. Institution: **Ranchi University, Ranchi, Jharkhand, India**

4. Date of Birth: **30 January 1986**

5. Gender (M/F/T): **Male**

6. Category Gen/SC/ST/OBC: **OBC**

7. Whether differently abled (Yes/No): **No**

8. Academic Qualification (Undergraduate Onwards)

	Degree	Year	Subject	University/Institution	% of marks
1.	**B.Sc.**	**2009**	**Botany**	**Ranchi University, Ranchi, India**	**65.13**
2.	**M.Sc.**	**2011**	**Botany**	**Ranchi University, Ranchi, India**	**74.25 (Gold Medalist)**
3.	**P.G.D.M.P.**	**2012**	**Ethnobotany**	**Ranchi University, Ranchi, India**	**78.80**
4.	**Ph.D.**	**2019**	**Plant Biotechnology**	**TERI School of Advanced Studies, New Delhi, India**	**Awarded**

9. Ph.D thesis title: **Development of transgenic lines of tomato and chilli plants against anthracnose disease**

Guide's Name: **Dr. Pallavolu Maheswara Reddy**

Institute/Organization/University: **TERI School of Advanced Studies, New Delhi, India**

Year of Award: **2019**

10. Work experience (in chronological order).

S.No.	Positions held	Name of the Institute	From	To	Pay Scale

11. Professional Recognition/ Award/ Prize/ Certificate, Fellowship received by the applicant.

S.No	Name of Award	Awarding Agency	Year
1.	**Gold Medal**	**Ranchi University, Ranchi, India**	**2011**
2.	**Teaching Assistantship Award**	**Ranchi University, Ranchi, India**	**2012**
2.	**DST-INSPIRE Junior Research Fellowship**	**Department of Science and Technology, Government of India, New Delhi, India**	**2013**
3.	**DST-INSPIRE Senior Research Fellowship**	**Department of Science and Technology, Government of India, New Delhi, India**	**2015**
4.	**Best Poster Award**	**Indian Phytopathological Society, New Delhi, India**	**2016**
5.	**Best Speaker Award**	**Nineteenth International Conference on Molecular Plant-Microbe Interactions, Osaka, Japan**	**2017**
6.	**Travel Grant**	**Department of Biotechnology, Govt. of India**	**2017**
7.	**Young Researcher Award**	**Institute of Scholars**	**2021**

12. Publications *(List of papers published in SCI Journals, in year wise descending order).*

S.No.	Author(s)	Title	Name of Journal	Volume	Page	Year
1.	Sagar S Arya, **Binod Kumar Mahto**, Meenu Sengar, Jim E Rookes, David M Cahill, Sangram Lenka	Metabolic engineering of rice cells with vanillin synthase gene (*VpVAN*) to produce vanillin	*Molecular Biotechnology, Springer Nature*	-	-	2022 (Accepted)
2.	**Binod kumar Mahto**, Anjulata Singh, Manish Pareek, M V Rajam, Swatismita Dhar-Ray, P M Reddy	Host-induced gene silencing of *conidial morphology 1* gene (*CgCOM1*) to confers resistance against anthracnose in chilli and tomato	*Plant Molecular Biology, Springer Nature*	104	381–395	2020
3.	Sagar S Arya, **Binod Kumar Mahto**, Thakku R Ramkumar, Sangram K Lenka	Sharpening gene editing toolbox in Arabidopsis for plants	*Journal of Plant Biochemistry and Biotechnology, Springer Nature*	29	769–784	2020
4.	**Binod kumar Mahto**, Poonam Sharma, M V Rajam, P M Reddy, Swatismita Dhar-Ray	An efficient method for *Agrobacterium*-mediated genetic transformation of chilli pepper (*Capsicum annuum* L)	*Plant Physiology Reports (Previously Indian Journal of Plant Physiology), Springer Nature*	23	573-581	2018

13. Detail of patents.

S.No	Patent Title	Name of Applicant(s)	Patent No.	Award Date	Agency/Country	Status

14. Books/Reports/Chapters/General articles etc.

S.No	Title	Author's Name	Publisher	Year of Publication
1.	Small RNA technology for plants abiotic stress tolerance	**Binod kumar Mahto**, Amit Katiyar, Sangram K Lenka, Kailash C Bansal	*Elsevier*	2020
2.	Mutagenomics for Functional Analysis of Plant Genome Using CRISPR Library Screen	Nidhi Dongre, Divyani Kumari, **Binod Kumar Mahto**, Sagar Sanjay Arya, Sangram Keshari Lenka	*Springer Nature*	2021

15. Any other Information (maximum 500 words):

I have master (M.Sc.) degree in Botany with specialization in Plant Physiology, Growth Development and Plant Biotechnology (**Gold Medalist**). During my studies, I became very interested in physiological phenomenon in plants and interaction of pathogens (microbes) to the plant. In the context of crop protection, during my final year of master degree, I worked on "Tissue culture studies in *Dhatura innoxia*". Then, I awarded by DST-INSPIRE Junior Research Fellowship (2013-2015) and DST-INSPIRE Senior Research Fellowship (2015-2018) from Department of Science and Technology, Govt. of India, New Delhi, India for pursuing Ph.D.

I awarded my Ph.D. degree (July 2019) on the crop improvement and protection of chilli and tomato plants against the anthracnose disease caused by an Ascomycetes fungus *Colletotrichum* spp., and my doctoral dissertation entitled "**Development of transgenic lines of tomato and chilli plants against anthracnose disease**" under the guidance of **Dr. Pallavolu Maheswara Reddy** (Senior Fellow, Sustainable Agriculture Division, The Energy and Resources Institute, New Delhi, India).

During my doctoral dissertation work was focused on development of resistant lines of chilli and tomato plants against anthracnose disease caused by *Colletotrichum* spp. by using the RNAi technique. So, we identified a specific gene (*COM1*) from *C. gloeosporioides*, and assessed the potential of Host-Induced Gene Silencing (HIGS) approach to target the *Colletotrichum gloeosporioides COM1* (*CgCOM1*) gene involved in fungal conidial and appressorium development, to restrict the fungal infection in chilli and tomato. For this study,

initially we standardized the *Agrobacterium*-mediated transformation procedure for chilli and tomato crops. Later, we developed stable transgenic lines of chilli and tomato using the RNAi construct of the fungal pathogen gene *CgCOM1* employing *Agrobacterium*-mediated transformation. Transgenic plants were characterized by molecular and gene expression analyses. Presence of specific *CgCOM1* siRNA in the transgenic chilli and tomato RNAi lines was confirmed by stem-loop RT-PCR. *C. gloeosporioides* challenge assays on leaves and fruits showed that the transgenic lines were resistant to anthracnose disease-causing *C. gloeosporioides* in comparison to the wild-type and the empty vector control plants. RT-qPCR analyses revealed barely any *CgCOM1* transcripts in the *C. gloeosporioides* infected tissues, indicating near complete silencing of *CgCOM1* gene expression in the pathogen. Microscopic studies on the *Cg*-challenged leaves of chilli-*CgCOM1*i lines showed highly reduced infection due to suppression of conidial germination, germ tube development, appressoria formation and mycelial growth in *C. gloeosporioides.* These results demonstrate that HIGS can be used to silence the expression of fungal developmental genes to inhibit the growth of disease causing pathogens, thus providing a precise approach to arrest the spread of disease and enhance food security.

I attended and presented our research work in four international conferences, and received best poster award (Poster presentation) and best speaker award (Oral presentation).

Undertaking by the Principal Investigator

To

The Secretary
SERB, New Delhi

Sir

I DR. BINOD KUMAR MAHTO herby certify that the research proposal titled "Tissue culture, Omics analysis and bioactivity Studies of Alpinia Spp." submitted for possible funding by SERB, New Delhi is my original idea and has not been copied/taken verbatim from anyone or from any other sources. I further certify that this proposal has been checked for plagiarism through a plagiarism detection tool i.e. TURNITIN approved by the Institute and the contents are original *and not copied*/taken from *any one or* many other sources. I am aware of the UGCs Regulations on prevention of Plagiarism i.e. University Grant Commission (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulation, 2018. I also declare that there are no plagiarism charges established or pending against me in the last five years. If the funding agency notices any plagiarism or any other discrepancies in the above proposal of mine, I would abide by whatsoever action taken against me by SERB, as deemed necessary.

BMahto 22.02.2022

Signature of PI with date

Name / designation

Dr. Binod Kumar Mahto
Assistant Professor
University Deptt. of Botany
Ranchi University, Ranchi

झारखण्ड सरकार

Office of the Deputy Commissioner, HAZARIBAGH
FORM OF CASTE CERTIFICATE TO BE PRODUCED BY EXTREMELY BACKWARD CLASSES/BACKWARD CLASSES APPLYING FOR APPOINTMENT TO THE POSTS UNDER THE GOVERNMENT OF JHARKHAND

Registration No. : JHCC/2017/474368 **Date: 06/05/2017**
Certificate No. : JHCC/2017/474368 **Issue Date: 12/05/2017**

This is to certify that Shri BINOD KUMAR MAHTO Son of Shri MANBODH MAHTO resident of GOVINDPUR KALA, Block - BISHNUGARH Gram Panchayat - GOVINDPUR KALA Village - Gobindpur Kalan Police Station - BISHNUGARH, Post Office - GOVINDPUR KALA, District - HAZARIBAGH, Jharkhand is a member of kurmi Community which is recognized as backward class under: Backward Class (Annexure - I) of Jharkhand Reservation of Vacancies and Posts (for Scheduled Castes, Scheduled Tribes and Other Backward Classes) Act - 2001* and professes Hinduism .**

This is also to certify that he does not belong to the Persons / Sections (Creamy Layer) mentioned in Column 3 of the Schedule to the OM No. 36012/22/93-Estt(SCT) dated 08.09.1993 of Department of Personnel and Training, Government of India as adopted by the Department of Personnel, Administrative Reforms and Official Languages vide Resolution No-3482 dated 10.06.2002.

1. * Caste / Sub Caste enumerated in Jharkhand Reservation of Vacancies and Posts (for Scheduled Castes, Scheduled Tribes and Other Backward Classes) Act - 2001.

2. ** The castes included in the list of Extremely Backward Classes/Backward Classes under Section-2 of the Jharkhand Reservation of Vacancies and Posts (for Scheduled Castes, Scheduled Tribes and Other Backward Classes) Act - 2001 vide Resolution No. 3885, dated 05.11.2011, 801 dated 11.02.2003, 3436 dated 28.06.2004, 6337 dated 08.12.2004, 6374 dated 11.12.2004, 368 dated 19.01.2006, 2759 dated 01.06.2006, 3706 dated 15.07.2006, 4447 dated 24.08.2007, 5182 dated 26.09.2006, 1604 dated 28.03.2007, 243 dated 11.01.2008, 5108 dated 23.09.2008, 4450 dated 01.08.2011, 5826 dated 19.09.2011, 6987 dated 26.09.2011, 6580 dated 20.10.2011, 8060 dated 17.12.2011, 144 dated 06.01.2012, 2855 dated 27.03.2012 and as revised from time to time.

Digitally signed by Authorized Signatory

Place : HAZARIBAGH
Date: 12/05/2017

"This certificate has been generated and digitally signed by electronic system.It is approved by authorized employee of related office"
This certificate is valid for one year from date of issue.

Endorsement Certificate from the Host Institute

This is to certify that:

I. The applicant **Dr. Binod Kumar Mahto** is working as **Assistant Professor, University Department of Botany, Ranchi University, Ranchi** (designation)* in this Institute. He/She joined the institution on date- **18 November 2020** (date - DD Month YYYY). We endorse his/her participation in the Project titled:**Tissue culture, omics analysis and bioactivity studies of *Alpinia* spp.**

II. The applicant is in regular position as defined by the term "Regular" in SRG guidelines.

III. The applicant will assume full responsibility for implementing the project as PrincipalInvestigator.

IV. The date of start of project is on the day when the Institutionreceivesthe first release of grant by RTGS transfer.

V. Thegrant-in-aidbytheScience&EngineeringResearchBoard(SERB)willbeusedtomeetthe expenditure on the project and for the period for which the project has been sanctioned as indicated in the sanction letter/order.

VI. No administrative or other liability will be attached to the Science & Engineering Research Board (SERB) at the end of the ResearchAward.

VII. The Institution will provide basic infrastructure and other required facilities to the investigator for undertaking the researchobjectives.

VIII. The Institutionwill take into its books all assets received under this sanction and its disposal would be at the discretion of Science & Engineering Research Board(SERB).

IX. The Institutionwill assume to undertake the financial and other management responsibilities of theproject.

X. The Institutionshall settle the financial accounts to the SERB as per the prescribed guidelines within three months from the date of termination of the ResearchAward.

26.02.22

Dated:

Signature of the Head of Institution

REGISTRAR
RANCHI UNIVERSITY, RANCHI

Seal of Institution

32652

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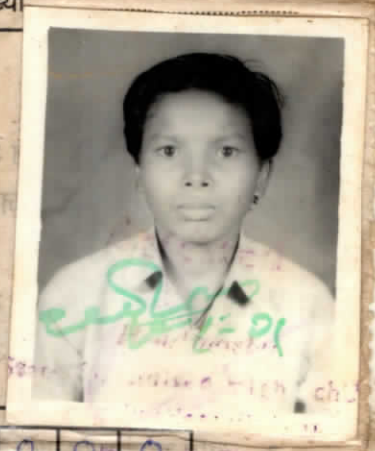
झारखंड माध्यमिक परीक्षा परिषद, राँची

परीक्षा प्रवेश पत्र

संख्या



1. परीक्षार्थी (हिन्दी में) विनोद कुमार महतो
   का नाम (अंग्रेजी में) BINOD KUMAR MAHTO
2. पिता का नाम : मनबोध महतो
3. जन्म तिथि : 30 (तिथि) 01 (माह) 1986 (वर्ष)
4. पंजीयन संख्या : 0212/018/12430/00 पंजीयन वर्ष 2000
5. परीक्षा : वार्षिक/पूरक
6. परीक्षार्थी : छात्र/छात्रा
7. जिला : हजारीबाग
8. अनमण्डल : हजारीबाग सदर
9. राष्ट्रीयता : भारतीय
10. धर्म : हिन्दू
11. पहचान चिन्ह : दाहिने हाथ में तिल का निशान
12. विषय (क) अनिवार्य विषय :
    - आधुनिक भारतीय भाषा : हिन्दी
    - द्वितीय भारतीय भाषा : संस्कृत
    - गणित
    - इतिहास
    - भूगोल
    - नागरिक शास्त्र
    - भौतिकी
    - रसायन शास्त्र
    - जीवविज्ञान
    - समाजोपयोगी उत्पादक कार्य (ग्रुप 1) एवं शारीरिक शिक्षा
    - अंग्रेजी

    (ख) अनिवार्य एच्छिक विषय : अर्थशास्त्र

विद्यालय प्रधान का हस्ताक्षर एवं मुहर 10-1-01

परीक्षार्थी का हस्ताक्षर

S.S. High School, Mandu Hazaribagh

(समिति कार्यालय के व्यवहार के लिए)

11. परीक्षा केन्द्र का नाम 132
12. रौल कोड क्रमांक 97

राजिक उ० वि० मुफ्फ

13. परीक्षा प्रारंभ की तिथि : 19 MAR 2001

सचिव
झारखंड माध्यमिक परीक्षा परिषद, राँची

Certificate from the Investigator

Project Title: Tissue culture, omics analysis and bioactivity studies of *Alpinia* spp.

It is certified that

1. The same project proposal has not been submitted elsewhere for financial support.
2. We/I undertake that spare time on equipment procured in the project will be made available to other users.
3. We/I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. We/I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in toto.
4. We/I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involves field trails/experiments/exchange of specimens, human & animal materials etc.
5. The research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.
6. We/I agree to abide by the terms and conditions of SERB grant.

Name and signature of Principal Investigator: **Dr. Binod Kumar Mahto**

Date: 22.02.2022

Place: Ranchi

Dr. Binod Kumar Mahto
Assistant Professor
University Deptt. of Botany
Ranchi University, Ranchi

Name and signature of Co-PI (s) (if any):

Date:

Place: