DBT-North East Centre for Agricultural Biotechnology

Assam Agricultural University

Jorhat, Assam

Minutes of 1st Scientific Advisory Committee (SAC) Meeting

Date: 21st-23rd September 2023

Venue: Meeting Room, DBT-NECAB New Building, AAU, Jorhat

Members present:

- Dr. T. J. V. Higgins (Honorary Fellow, CSIRO, Canberra): Chairman, SAC
- Dr. Bidyut Chandan Deka, Honorable Vice Chancellor, AAU: Co-Chairman, SAC
- Dr. M. Aslam, Retd Adviser, DBT, GoI; Consultant DBT-ILS: Member
- Dr. T. Madhan Mohan, Consultant Adviser, DBT-APSCS&T Skill Vigyan Kimin, Arunachal Pradesh: Member (joined online)
- Dr. N. K. Singh, ICAR-National Professor (B. P. Pal Chair) and Ex Director NIPB: Member
- Dr. M. V. Deshpande, Director, Greenvention Biotech, Pune: Member
- Dr. Utpal Borah, Professor, IIT Guwahati: Member
- Dr. Jayanta Deka, Dean, FA, AAU, Jorhat: Member
- Dr. Mrinal Saikia, Director of Research (Agri): Member
- Dr. Anup Kumar Das, Director of Post Graduate Studies, AAU
- Dr. M. K. Modi, Retired Professor and former Head of Department (HoD), Agricultural Biotechnology, AAU: Member
- Dr. B. K. Sarmah, Director and current HoD, DBT-NECAB: Member, Secretary

All PIs, Project Scientists, Research Associates and Ph.D. Scholars were also present during presentations by the PIs.

The Scientific Advisory Committee (SAC) meeting of the DBT - North East Centre for Agricultural Biotechnology (DBT-NECAB) was held at the new DBT-NECAB Building, Assam Agricultural University (AAU), Jorhat, from September 21st to 23rd, 2023. The programme commenced with the playing of the AAU anthem, followed by a welcome address delivered by Dr. B. K. Sarmah, Director of DBT-NECAB. Dr. Sarmah cordially welcomed Dr. T. J. V. Higgins, Chairman of the Scientific Advisory Committee, and Dr. T. Madhan Mohan, who were in attendance via online mode. Other attendees included esteemed experts from DBT, namely Dr. M.

Aslam, Dr. N. K. Singh, Dr. M. V. Deshpande, Dr. Utpal Borah, Dr. J. Deka, Dr. Mrinal Saikia and Dr. Anup Kumar Das. Dr. Sarmah started the programme with his concise exposition by expressing appreciation to the Department of Biotechnology (DBT) for the substantial financial assistance provided, which significantly facilitated the establishment of the new DBT-NECAB edifice. The building was ceremoniously inaugurated in a virtual manner by the esteemed Chief Minister of Assam, Dr. Himanta Biswa Sarma, accompanied by the Vice Chancellor of AAU, Dr. Bidyut Chandan Deka and other prominent people associated with the institution, on the 16th of August 2023.

In his presentation, Dr. B. K. Sarmah emphasized on the progress and achievements of all five R&D programmes and academic activities of the Centre. In brief, Dr Sarmah reported development of two new rice varieties; one tolerant to bacterial blight which was already notified and names as Patkai; another tolerant to drought, which is in the processes of multilocational trial. Furthermore, development of introgressed Bt chickpea lines at PSAU, Ludhiana and UASD, Karnataka; identification of genes for acid tolerance; development of novel bioformulations by the Centre were also reported. The programme was thereafter accompanied by the acknowledgement and recognition of the esteemed visitors.

The inaugural address was delivered by Dr. T. J. V. Higgins, Honorary Fellow at CSIRO in Canberra. As the Chairman of SAC, Dr. Higgins expressed his appreciation to all the attendees of the programme and acknowledged his twelve-year connection with DBT-NECAB. Furthermore, he extended congratulations to the members of both DBT-NECAB and AAU for their significant accomplishments.

Dr. M. Aslam, retired adviser of the Department of Biotechnology (DBT), GoI, expressed his congratulations to Dr. Sarmah on the successful completion of the construction of the new facility for DBT-NECAB. Additionally, he extended congratulations to Dr. Sarmah for the successful registration of two bioformulations from Programme IVb of DBT-NECAB in Central Insecticides Board (CIB).

Dr. T. Madhan Mohan, Consultant Adviser at DBT-APSCS&T Skill Vigyan Kimin, expressed admiration for the notable achievements of DBT-NECAB over the last decade which embrace several areas including research, product development, extension activities, multilocation field trials of genetically modified chickpea varieties, and outreach programmes.

Dr. N. K. Singh, ICAR, National Professor, said that he has been involved in DBT-NECAB for 10 years. He also commended the whole DBT-NECAB fraternity on their tremendous research output, the construction of satellite labs in various regions of the North-East India, and the efforts of those responsible scientists and Dr. Sarmah in successfully registering bioformulation with CIB. He also appreciated the tremendous financial assistance provided by the DBT, GoI, to DBT-NECAB in the past decade, which has been critical in achieving such progress over a ten-year period.

Dr. M. V. Deshpande, Director of Greenvention Biotech Pvt. Ltd., complimented the scientists and research scholars associated with DBT-NECAB for their outstanding success in publishing a significant number of research publications in prestigious peer-reviewed journals. He and Dr. Utpal Borah, Professor at IIT Guwahati, expressed satisfaction for the centre's remarkable efforts in efficiently transferring technologies from laboratories to the farmers' fields, hence improving the agricultural community's overall welfare.

Dr. Jayanta Deka, Dean, College of Agriculture, AAU, Dr. Anup Kumar Das, Director of Post Graduate Studies, AAU and Dr. Mrinal Saikia, Director of Research (Agri), AAU, Jorhat all congratulated the Director, DBT-NECAB and all the scientists and research scholars associated with the centre for the significant achievement made in the last ten years in terms of research outcomes, product development, technology transfer through extension activities and sharing of the facilities of DBT-NECAB with other regional institutions. They also put special emphasis on the encompassment of different disciplines with the centre.

During the session, the manual titled "Microbiological Techniques for Mass Production of Plant Beneficial Microbes" and the bulletin titled "Microbes in Precision Agriculture" were also made available. The first session was concluded with a vote of thanks delivered by Dr. Madhumita Barooah, Professor, Department of Agricultural Biotechnology, AAU.

The subsequent session began with a thorough review of the specifics outlined for each programme. The principal investigators (PIs) provided an update on the progress of their work through PowerPoint presentations. The suggestions provided by the members of the Scientific Advisory Committee (SAC) are outlined and discussed below:

PROGRAMME I

Genetic improvement of rice for biotic and abiotic stress tolerance using molecular breeding, especially drought, submergence, bacterial blight, and blast diseases

Dr. Sanjay Kumar Chetia, AAU-ARRI

Dr. Priyabrata Sen, AAU

Presented by: Dr. Sanjay Kumar Chetia

Progress:

- Assam Rice Association Mapping (ARAM) panel with 300 entries has been developed and seeds multiplication has been initiated.
- For identification of QTLs/genes associated with submergence stress tolerance, two bi-parental mapping populations were developed by crossing No. 89 and No. 135 with Numoli and F_3 seeds were raised for generation advancement**.**
- To develop population for Bacterial Leaf Blight (BLB) and Blast resistance, an improved Samba Mahsuri line (MSM-BLBB) consisting of three genes for BLB resistance and two genes for Blast resistance was crossed with BRRI-75, Shiva, DRR-45, No. 29, Disang and Ranjit Sub-1 and seeds from F_3, F_5, F_6, BC_1F_2 and BC_2F_2 generation were sown during *Kharif-2023* and foreground selection with targeted gene-based markers will be conducted.
- Kolong x IR64 drought selected introgression line No. 8 carrying three drought tolerant QTLs (*qDTY1.1, qDTY2.2* and *qDTY4.1)* has been forwarded for multi-location trials (MLT) in all the agro-climatic zones of Assam.
- A Bacterial Blight resistant introgressed rice variety 'PatKai' has been released in Assam. This variety was notified by CVRC in the year 2023, notification No. 3-85/2023-SD.IV dated 9th June, 2023.
- The introgressions of drought tolerant QTLs (*qDTY1.1, qDTY2.2* and *qDTY4.1)* from 'IR64-drought' into a submergence tolerant variety 'Ranjit Sub-1' has been started and F_2 seeds were sown during Kharif-2023.

Four scientific studies were published under this programme during the period of 2022-2023.

Suggestions SAC members

- The SAC members were very impressed with the progress made under this programme.
- Dr. Aslam commented that it was a nice example of translational work and pooling of resources, expertise and material that is the key to success of any translational work.
- Dr. N. K. Singh congratulated the team working on this project for their excellent work as one new rice variety resistant to BLB has been notified and will be used in the farmers' field.
- Dr. Singh suggested that since the DBT-NECAB programme has enough resources now, it is the perfect time to consider the use genome editing (SDN1 and SDN2) for crop improvement against diseases.
- Dr. Modi proposed that Jorhat centre should focus on genome editing and Titabar centre is doing very good at field work so lab work should be done at Jorhat centre.
- Dr. Upal Borah praised research activities under this programme as they are relevant with the current climate change scenario.
- Dr. M. Aslam asked for a brief note on the variety "Patkai", which can be sent to DBT along with the good quality photographs to promote and create awareness among the farmers through social media.

PROGRAMME II

Development of insect resistant chickpea varieties protected against *Helicoverpa armigera*

Dr. B. K. Sarmah, (Project Coordinator)
Dr. Sumita Acharjee (Principal Investigator)

Collaborators:
Dr. Shyla Bindra (PI) (PAU, Punjab)
Dr. Bagewadi Basavaraj (PI) (UAS, Dharwad)
Dr. T.J.V. Higgins (PI) (CSIRO, Australia)

Presented by: Dr. Sumita Acharjee

Objectives of phase III:

- Biosafety analyses of chickpea Cry1Ac and Cry2Aa lines in field condition
- Molecular characterization of available Cry2Aa lines for selection of one line for full T-DNA analysis including flanking regions.
- Crossing of selected homozygous Cry1Ac and Cry2Aa lines for pyramided events.
- Generation of transgenic chickpea lines expressing a *vip3A* gene for *Helicoverpa* resistance.

Dr. Acharjee has communicated to the members of the Scientific Advisory Committee (SAC) that event selection trials (ESTs) will be carried out for both the Cry1Ac and Cry2Aa lines in Phase-III of the DBT-NECAB. These trials will be conducted in conjunction with Biotech Consortium Pvt. Ltd. (BCIL).

Progress made under Programme II:

- An application has been submitted to the Review Committee on Genetic Manipulation (RCGM) to obtain consent for conducting ESTs at Punjab Agricultural University (PAU) and University of Agricultural Science (UAS), Dharwad. The ESTs will be conducted two events with Cry1Ac gene (100B and 80G) and two events with Cry2Aa gene (72C2 and 80C). The introgressed lines developed using such lines at PAU and UASD will also be included.

- A total of 86 transgenic chickpea lines have been successfully developed, each expressing the Cry1Ac gene. From this pool, two lines have been chosen for further evaluation in event selection trials, based on their level of target gene expression.
- The quantification of Bt proteins in various organs of the chickpea plant has also been assessed, as this information is essential when completing an application for obtaining consent from the RCGM. A greater accumulation of Bt protein has been observed in the leaves and flowers, while the concentration of Bt protein in the dry seed was found to be minimal. The transgenic chickpea line 100B is devoid of any detectable levels of NPT II protein, which serves as a selectable marker gene, in its dry seed. The 100B and 100E lines also lack any integrated vector backbone sequence in the chickpea, which is crucial from a regulatory perspective.
- The proximate analyses performed on the Bt chickpea seeds did not show any noticeable differences in contrast to their non-transgenic counterparts in terms of starch content, reducing sugar levels, crude fat composition, nitrogen content, crude protein concentration, and ash content. The chickpea line 100B, when compared to its non-transgenic counterparts, did not exhibit any statistically significant variations in terms of fatty acid content, minerals, vitamins, flavones, or anti-nutrient factors. The digestibility pattern of total seed proteins in Bt chickpea lines was found to be comparable to that of the non-transgenic lines when subjected to in vitro digestion.
- Nanopore sequencing has already been performed to ascertain the flanking sequences within which the T-DNA has been integrated into the genome of chickpea, leading to the identification of two methyl transferase genes, specifically COMT (caffeic acid 3-O-methyltransferase (COMT)-like gene) and ANMT (Anthranilate-N-methyltranferase-like gene). Event-specific PCR was carried out by designing primers based on sequence information of the RB and flanking regions. This analysis indicated that the integration of the Cry1Ac gene between COMT and ANMT did not have an impact on the expression levels of these two genes in both transgenic and non-transgenic lines.
- A total of 150 transgenic chickpea lines containing the *cry2Aa* gene have been successfully developed. Among these lines, two specific lines, namely 72C2 and 80C, were identified as lines with single copy of transgene and demonstrated a notable increase in the expression

of the Bt protein. The recorded insect mortality rates for 72C2 and 80C were 74% and 100%, respectively.

- Several new transgenic lines have been developed in the last two years that express the full-length *cry1Ac* gene, instead of the truncated version. These lines are specifically named 83A, 183C, 82A, 182D, and 91A. Among the five transgenic lines, it was observed that line 91A exhibited a significantly higher accumulation of the Bt protein, with a concentration of 51 µg/g FW.

Milestones to be accomplished:

- Dietary exposure of transgenic proteins (Cry1Ac and nptII protein) to humans and livestock in collaboration with the National Institute of Nutrition (NIN), Hyderabad.
- Digestibility and oral toxicity of Cry1Ac and nptII (protein safety analysis), in collaboration with CSIR- Indian Institute of Toxicology Research, Lucknow.
- The proximate analyses of seeds, including the estimation of minerals, vitamins, and other components, are to be conducted at the Department of Agricultural Biotechnology (ABT) and Department of Agricultural Biochemistry (BAC) of AAU, Jorhat.
- Environmental risk assessment research will be conducted in partnership with PAU and UAS, Dharwad to examine the weediness of transgenic events, lateral gene flow to other species, and impact on non-target organisms.
- Gene pyramiding, or the introduction of new genes, to increase the resistance of transgenic chickpea to specific insect species. To achieve this objective, following strategies will be employed. The first is a transgenic approach; the second takes advantage of flanking sequence information from another transgenic line, 39C (stable transgenic line), which contains the *α-amylase inhibitor* gene on chromosome 4 between zeatin o-glucosyltransferase and benzyl alcohol o-benzoyltransferase (flanking region on the left) and benzyl alcohol o-benzoyltransferase (flanking region on the right), respectively. This involves utilising the CRISPR/Cas-mediated genome editing tool (SDN3) to effectively integrate the *vip3* gene into chromosome 4, employing *vip3* as the donor DNA.

Compliments given:

- Dr. N. K. Singh commended the Principal Investigator (PI) of the programme and its collaborators for their expertise in conducting comprehensive molecular analyses, such as event-specific PCR. This is particularly noteworthy as many scientists from various laboratories lack a thorough understanding of the intricacies involved in molecular analyses of transgenic events.
- Dr. M. Aslam congratulated the principal investigator (PI) and the team for their successful advancement of genetic modification research in chickpeas. This achievement is noteworthy considering that similar research initiatives were undertaken in various laboratories and institutes across the country, supported by different funding agencies. Nevertheless, many of these efforts did not achieve the same level of success as the PI and collaborators of this programme. He extended his congratulations to the entire team of this programme for their consistent effort, hard work, and dedication over a significant period of time to bring the programme to its current stage.

Questions raised:

- Dr. N. K. Singh inquired Dr. Acharjee about the availability of containment facilities at AAU, Jorhat for conducting event selection trials. In her answer, Dr. Acharjee said that the event selection trials would not be performed at AAU. Instead, it will be undertaken in partnership with PAU and UAS, Dharwad, using the facilities they have for the purpose of conducting such investigations.
- Dr. N. K. Singh additionally pointed out that the adoption of the SDN3 approach, which involves introducing the *vip3* gene into transgenic lines containing the *α-amylase* gene, may raise regulatory concerns when seeking approval from the RCGM and other biosafety authorities for conducting field trials.

PROGRAMME III

Elucidation of acid and its associated stress tolerance mechanisms in soil bacteria: Towards increasing the efficiency of agriculture production in acid soils of Northeast India

Dr. Madhumita Barooah (PI)

Dr. Robin Boro

Presented by: Dr. Madhumita Barooah

Progress

- Genes associated with proline and arginine biosynthesis and protein-protein interaction have been identified in *Priestia megaterium* through bioinformatics analysis.
- Acid tolerance genes related to proline biosynthetic pathways have been identified and the PPI network has been generated.
- To check the expression of these genes in wild type and the ΔproC mutant a qRT-PCT was performed in different conditions.
- As per the suggestion of previous SAC meeting, transformation process of acid tolerance gene from bacteria to the model plant has been initiated.
- pH study on the growth of model plant at different pH to check the behavior of model plant at different pH conditions is under process.
- Whole genome sequence analysis of *P. megaterium* G18 has been performed, and the genes involved in acid tolerance and general stress response have been identified.
- In the next study, effect of iron toxicity has been checked in different rice cultivators under acid stress conditions and phylogenetic tree analysis of different iron tolerant and sensitive rice cultivators under acid stress conditions have been generated.
- Similarly, effect of aluminium toxicity has been checked in different rice cultivators under acid stress conditions and phylogenetic tree analysis of different aluminium tolerant and sensitive rice cultivators under acid stress conditions have been generated.
- Multilocational trial for evaluation of promising plant growth promoting bacteria (PGPB) for growth and yield in rice has been initiated.

- Biopriming of lentil seeds with acid tolerant PGP bio inoculum demonstrates that Consortium and vermicompost treatment showed the highest grain yield and nodule number in lentils.
- Experiments to enhance the germination and establishment of hypoxia-sensitive rice plants using microbial inoculants are under process.
- Evaluation of promising PGPB for Promoting Anaerobic Germination in Direct Seeded Rice is under process.

Suggestions by SAC members

- Dr. Deshpande inquired about the rhizobium diversity in the soil of Assam. Dr. Madhumita Barooah conveyed to the members that the symbiotic relationship is not very well understood yet in the case of legumes. Her group is interested in studying how acidic soil impacts rhizobium nodulation.
- Dr. Deshpande additionally pointed out that rhizobium is important for phosphate solubilization. During this biochemical process, organic acids are produced. So how it can be tolerant for acidic conditions? Dr. M. K. Modi and Dr. Madhumita replied that nodulation in legumes is very poor in North-East regions, which accounts for the lower production of legume crops in this area compared to pulses. Dr. Deshpande replied that in Maharashtra, rhizobium is primarily used for phosphate solubilization in alkaline soil.
- Dr. Deshpande has raised concerns regarding the abundance of bacterial cultures present in this programme. What steps should be taken for moving forward? How can these organisms be utilized in fields? Dr. Madhumita explained that the release of the bioformulation will follow a sequential process, beginning with a local station trial, followed by a multi-location trial, and finally farmers' field trial. The AAU authority has now approved the field trial and accordingly trials will be conducted at various stations.
- Dr. B. K. Sarmah proposed that, prior to conducting field trials, we must conduct multilocation trials at six different sites. Once the performance from these trials has been evaluated, approval from the University will open the path for field trials.
- Dr. Sarmah mentioned that funds have been provided to Dr. Madhumita for the evaluation of PGP bio-inoculum to check growth and yields in crops. Dr. Madhumita replied that

multilocation trials for evaluation of PGPB have already been started, and her team is getting good results.

- Dr. Utpal Bora asked about the similarity between the plant and bacterial *proC* genes. Dr. Madhumita replied that both sequences are not very similar. Dr. Bora proposed to make changes in the *proC* gene in *Nicotiana tabacum* using gene editing technology and then introduce similar *proC* from a bacterial species with all the regulatory regions essential for its expression in the plant system. This method will allow the assessment of how plant growth is affected in an acidic environment.
- Dr. Sarmah added that if it can be demonstrated that genes from soil microbes responsible for acid tolerance can be transferred to the plant system, and then if the plant system shows acid tolerance, it would be a valuable study. Dr. Sarmah added that this suggestion was also given by the DBT-Scientific and Technical Appraisal and Advisory Group (STAG) committee.
- Dr. Deshpande suggested using the natural properties of bacterial isolates for formulating purposes because genetic modifications are not permissible in fertilizers as per regulations. Dr. Sarmah added that the guidelines for genetic modifications of bacterial isolated are still in a draft form, which was released in 2017.
- Dr. N. K. Singh mentioned that proline has been implicated in a variety of biotic and abiotic stress situations. There can be multiple mechanisms for acid tolerance. So, other factors such as proton pump, different transport proteins, and membrane proteins should be considered for experiments.
- Dr. M. Aslam proposed that he would connect Dr. Madhumita with a group in Gujarat that was inquiring about laboratories involved in acid tolerance research.

PROGRAMME IVa

Isolation of novel strains to develop efficient biofertilizers

Dr. Rajan Baruah (PI and Professor), Department of Soil Science, AAU, Jorhat

Presented by: Dr. Rajan Baruah

Objectives:

1. Effects of existing biofertilizers on rhizosphere microbiota and enzymatic processes; growth and yield of popular rice variety of Assam
2. Field demonstration and technology transfer
3. Isolation of new potent novel strains from different crop rhizospheres

Progress:

- On Farm Trial (OFT) was conducted at Tinsukia, Dibrugarh, Golaghat & Jorhat districts, to study the effects of existing biofertilizers on rhizosphere microbiota and enzymatic processes; growth and yield of popular rice variety "Ranjit sub-1".
- Biological parameters, such as plant height, tiller numbers, panicle length, yield, % increase over farmers practice, and B:C ratio, were evaluated. Variation in performance was observed w.r.t. spatial location.
- Biofertilizers application showed a positive trend in enhancing yield and yield attributing parameters. In addition, increased rice yield to the tune of 15.69 to 33.3% recorded in the farmer's field of four districts of Assam because of biofertilizer application.
- Crop improvement due to biofertilizer application reflected better in farmer's field of Tinsukia and Dibrugarh districts as compared to Jorhat and Golaghat districts.
- The soil samples were subjected to the chemical properties analysis. Properties such as pH, N (%), P_2O_5 (kg/ha), K_2O (kg/ha), O.C. (%), O.M. (%), B.R. were evaluated. Along with the chemical properties, biological parameters (Urease, Protease, Dehydrogenase, Catalase, Arylsulfatase, FDA, β-glucosidase, Phosphomonoesterase, Phosphodiesterase, and Phosphotriesterase) of soils from Tinsukia and Dibrugarh districts were also evaluated.
- Dr. Rajan's group observed that irrespective of treatment, all soil samples were acidic in nature. Among the treated samples, the levels of chemical components such as N, P_2O_5, and K_2O were consistently in the low to medium range. Interestingly, the percentage of

organic carbon (% O.C.) and organic matter (% O.M.) were recorded to be higher in the soils treated with biofertilizer and compost.

- Biofertilizer treatment recorded highest soil microbial activities in soil samples with respect to urease, protease, DHA, catalases and phosphatases.
- Efficient biofertilizer agents supplied to progressive farmers of Kardoiguri and Majuli for restoration of riverbank erosion.
- For isolation of beneficial microbes from different crop rhizospheres and endosphere, the crop specific rhizospheric soil and root samples collected from 23 different locations around Jorhat for isolation of beneficial microbes.
- Preliminary screening showed 17 isolates were nitrogen fixers and 15 cultures were phosphorus solubilizers. The screening of cultures for other nutrients transformation is under process.

Work in progress:

- Qualitative and quantitative assay of the plant growth promoting traits of the bacterial isolates.
- Microbial enzyme activities and soil biological properties
- Microscopic characterization of the bacterial isolates, microphotographs, and staining behavior
- Biochemical characterization of the bacterial isolates
- Molecular taxonomy of selected bacterial isolates
- Whole genome sequencing of the selected novel cultures.

Suggestions SAC members

- The SAC members praised the work done under this programme.
- Dr. Utpal Bora inquired about Ranjit sub-1 yield after treatment with biofertilizer. In response, Dr. Rajan explained that the yield varied across different locations, for example in Titabar the yield was higher compared to Jorhat.
- Dr. Deshpande asked about the unit of enzyme activity and suggested converting the unit of enzyme amount into enzyme activity.

- Dr. Utpal Bora and Dr. N. K. Singh supported Dr. Deshpande's suggestion and advised not to use enzyme quantity as a representation of enzyme activity.
- Dr. Singh suggested to include a control group in the rejuvenation programme conducted at the Brahmaputra riverbank. This step is essential to establish whether biofertilizers genuinely help crops in withstanding flood conditions.
- Dr. Utpal Bora suggested thinking about where the control group should be planted for experimental purposes.
- Dr. M. Aslam suggested the utilization of biofertilizers in vetiver cultivation because it has been recommended that cultivating vetiver in Majuli can contribute to economic development.
- Dr. B. K. Sarmah mentioned that farmers cannot use chemical fertilizers at the Brahmaputra riverbank so this can be a potential solution to the problem.
- Dr. Sarmah also suggested that from next time while biofertilizers used for Brahmaputra rejuvenation programme we must go there and design the experiments for scientific outcome.
- Dr. Deshpande asked about the inoculum load, and Dr. Rajan responded that it was 10^8 cfu.

PROGRAMME IVb

Isolation of novel microbial strains from NE region to develop efficient biopesticides

Dr. Popy Bora (PI), Senior Scientist, Plant pathology, AAU, Jorhat

Dr. Pradip Kr. Borah, Professor, Plant Pathology, AAU, Jorhat

Dr. Inee Gogoi (Co-PI), Scientist, Entomology, AAU, Jorhat

Presented by: Dr. Popy Bora

Objectives:

1. Scale up and demonstration of developed biopesticides
2. Identification of novel genes, generation of multi-locus DNA barcode sequences of new and important microbial strains
3. Bioprospecting novel microbial strains for bioremediation

Progress:

- A total of 23 microbial agents were registered with the National Repository ICAR-NBAIM, Mau and accession numbers were received. Registration of microbial agents is mandatory as per the guidelines. In addition, revival of culture is also possible from ICAR-NBAIM in case we lost our culture. Accession numbers for all the registered microbial agents were shown.
- Registration of two biopesticides (Bioveer-2 and Biomonas) is under pipeline. Most probably, in next 6-8 months certification can be obtained. Dr. Deshpande was acknowledged for his through guidance.
- Technology transfer to the Poohar FPC, College of Agriculture Tripura, and College of Agriculture Nagaland is under process.
- Most of the bioagents have shelf life of about 9 months. It is possible to enhance the shelf life by encapsulating the bioagents because it will not be in direct contact with the environmental conditions.
- Multilocation trials of *B. subtilis* based liquid formulation at different zonal stations under AAU are currently ongoing. For trials, MOU will be signed with the following institutes:

CA, Tripura; CA, Nagaland; University of Horticultural Science, Bagalkot; Greenvention Biotech, Maharashtra; RVSKVV, Gwalior.

- A total of 23.7 lacks revenue is generated form the laboratory products from 2019 to 2023.
- Khasi Mandarin is very prone to post-harvest diseases and unfortunately is overlooked. It is must to come with a solution to stop post-harvest disease. As of now only one bacillus-based product is available and that is too from the USA. Dr. Popy Bora's lab tried some of the bioagents to study their impact. The SEM imaging technique showed that *B. subtilis* LB22 interacts with both *Penicillium digitatum* and *Aspergillus niger*.
- LC-MS profiling of *B. subtilis* LB22 showed production of anti-microbial metabolites.
- After testing four Bacillus species against post-harvest pathogens of Khasi Mandarin, Dr. Popy Bora's group found that both the cell suspension and cell-free culture filtrate of *B. subtilis* exhibited a biocontrol efficiency of one hundred percent and could be utilised as a fruit coating technology to protect against the pathogens.
- Qualities such as colour, flavour, texture, and taste, are not affected after introducing *B. subtilis* based bioagents. Toxicology data suggests that it is not toxic to mammals, so this technology can be taken to next step.
- To establish a standardised technique for the encapsulation of bioagents, the efficacy of sodium alginate, sodium CMC, and Xanthan gum as coating agents was assessed. Experiments were done to assess the duration of storage durability of bioagents coated with microcapsules, as well as to investigate the degradation of these microcapsules when exposed to soil.
- Ongoing collaborations with different institutes were also mentioned in the presentation.

Suggestions SAC members

- Dr. Deshpande inquired about the rate of release of encapsulated biopesticide material and the shelf life of said material. Dr. Popy indicated that the release process begun after a period of 7-8 days. Furthermore, it was observed that the product was effective even in dry soil conditions. They are currently engaged in the estimation of shelf-life periods.
- Dr. Deshpande also asked about the load of bacteria in microcapsules. Dr. Popy Bora replied $x10^8$ cfu in case of bacteria, and for fungal strain the standard load was $x10^6$ cfu.

- Dr. Deshpande recommended focusing the research efforts on Bacillus strains as a potential solution for controlling storage market disease. This is because there is currently no product available to effectively mitigate the losses caused by such disease. This product holds significant importance.
- Dr. Utpal inquired about the temperature at which sodium alginate was used for encapsulation purposes. Dr. Popy Borah replied that sodium alginate was used at ambient temperature.
- Dr. M. Aslam suggested to make a research strategy on fungal diseases in crops and how to reduce fungicide residues. The regulatory agencies are insisting to work on this, so that problem related to storage and export can be solved.

All the SAC members praised the completed work and extended their congratulations to all the group PIs for their exceptional scientific contributions and dedicated efforts.

The meeting concluded with expressions of gratitude from the Chair.

DBT-North East Centre for Agricultural Biotechnology

Assam Agricultural University

Jorhat, Assam

Minutes of 1st Scientific Advisory Committee (SAC) Meeting

"Only SAC members"

Date: 23 September 2023

Venue: Meeting Room, DBT-NECAB New Building, AAU, Jorhat

Members present:

Only the members of the SAC meeting

Dr. T. Madhan Mohan, Consultant Adviser, DBT-APSCS&T Skill Vigyan Kimin, Arunachal Pradesh (joined online)

Dr. Mohammad Aslam, Retd. Adviser, DBT, Government of India; Consultant DBT-ILS

Dr. Nagendra Kumar Singh, ICAR-National Professor (B. P. Pal Chair) and Ex Director NIPB

Dr. Mukund Vinayak Deshpande, Director, Greenvention Biotech, Pune

Dr. Utpal Bora, Professor, Biosciences and Bioengineering, IIT Guwahati, Assam

Dr. Bidyut Kumar Sarmah, Director and current HoD, DBT-NECAB, AAU, Jorhat

Dr. B. K. Sarmah started the meeting:

Dr. Sarmah expressed his gratitude to all the SAC members for their valuable suggestions on the progress and achievements made under the DBT-NECAB programme. Dr. Sarmah also thanked the DBT for providing funds to carry out R&D programmes. Later, Dr. Sarmah requested the SAC members for suggestions on the R&D programmes, academic programmes, finance, administration, and overall management.

Suggestions from Dr. T. Madhan Mohan:

Dr. T. Madhan Mohan thanked Dr. B. K. Sarmah and all the SAC members. All the presentations and work done by principal investigators were appreciated. The new building of DBT-NECAB, collaboration with IIT Guwahati, and consistent connection of DBT-NECAB with north-east institutions such as satellite labs were also appreciated by Dr. T Madhan Mohan.

Dr. Madhan Mohan suggested exploring collaborative opportunities with both national and international agricultural institutions. The DBT-NECAB programme has established research facilities, techniques and products in a remarkably short time. Now it is the time to provide expertise to other institutions. This type of collaboration will provide a strong base for scientific research to other institutions, which can lead to major breakthroughs.

The DBT-NECAB programme should include rice varieties from other states in the North-Eastern region. In this way, the Assam Rice Association Mapping (ARAM) will not only strengthen Assam but also benefit other states in North-East India. This research will help in advancing rural development in this region.

There are ample opportunities for entrepreneurship in the form of startups. Agencies like BIRAC, DBT and DST conduct training programmes, provide funding, and help in translating research into products. Now it is the time to go for product development. Later, Dr. Madhan added that he was very happy with the successful commercialization of the biofertilizers and the development of bacterial blight resistant rice variety "Patkai".

Dr. Mohan added that collaborations with prominent institutions were essential for mutual benefits. With this DBT-NECAB can overcome any limitations and help other institutions with its expertise and resources. For strong collaborations, design the network programme in such a way that funding should also go to the collaborators.

Dr. Mohan put emphasis on regular discussions with project PIs and research scholars so that the resources can be used in a proper and timely manner.

At the end, Dr. Madhan Mohan thanked all the SAC members and the Director of DBT-NECAB for the wonderful discussion and suggestions during the meeting, which was held in the morning session.

Response of Dr. B. K. Sarmah:

The DBT-NECAB centre is already collaborating with national and international institutions. For example, CSIRO in Australia, and institutions in the USA and Switzerland. For new developments and objectives, it is important to go for more collaborations. Especially for the chickpea project, DBT-NECAB is collaborating with Punjab Agricultural University and University of Agricultural Science, Dharwad. Both ICAR and DBT-STAG suggested to incorporate more collaborators for transferring the transgenic chickpea lines for further evaluation and introgression breeding.

In response to the network programme, Dr. Sarmah replied that currently two network programmes are running, one on tea and second on citrus. Furthermore, one network on the improvement and management of horticultural crops is also under consideration by the DBT.

Suggestions from Dr. Deshpande:

The microbiology programme received appreciation for its accomplishments. According to Dr. Deshpande, the main concern is that this research is not being used for social welfare. For this, the programme leader should give cultures to farming community for the betterment in the form of biopesticide and biofertilizers.

Overall, Dr. Deshpande found that DBT-NECAB is a knowledge-based programme. Everyone has shown great motivation in their respective area of research. This will certainly lead the project leaders to translate their research efforts into technology and product development. At the end, Dr. Deshpande extended his best wishes.

Suggestions from Dr. N. K. Singh:

The progress and number of quality publications were appreciated. It is a great news that GM Chickpea from DBT-NECAB programme is currently undergoing RCGM evaluation process for the event selection trial approval. Dr. Singh added that this programme uses cutting edge technologies, for example next-generation sequencing and event-specific PCR techniques. Now this centre is one of the leading centres in India. It is now well-proved by the evidence that people from leading institutions such as National Institute for Plant Biotechnology (NIPB) and others are coming for training programme at AAU, Jorhat.

The new building of DBT-NECAB is very thoughtfully designed. The interior, research labs, and auditorium, all were designed perfactly.

Dr. Singh suggested that genome editing technique should be applied as soon as possible for rice improvement programme Dr. Singh added that genetic modification can also be applied to microbes. At the end, Dr. Singh extended his best wishes.

Suggestions from Dr. Utpal Bora:

Dr. Bora congratulated Dr. Sarmah for his excellent leadership at DBT-NECAB. The project leaders and their entire team were also praised for their excellent work.

The impact of DBT investment is evident through the excellent research and infrastructure at AAU, Jorhat. The quality of publications and resources were appreciated. He added that care needs to be taken of resources such as collected microbial strains and generated Chickpea lines. These important resources can be used to develop partnerships with other institutions. For example, a knockdown proC line of *Bacillus megaterium* G18 can be helpful for other institutions. Dr. Bora also supported the suggestion given by Dr. Mohan that a greater number of rice varieties, especially from the northeastern hill areas, should be included for QTL identification and mapping so that the entire North-East region can get benefited by the research conducted at AAU, Jorhat. For doing this, help from satellite labs can be taken.

There should be an internal meeting every three months. It will not only help in tracking progress and improving methods but also lead to identifying gaps in the research, which can be filled with the help of other experts. At the end, Dr. Bora expressed his thanks to Dr. Sarmah for conducting a very nice and well-organized meeting.

Suggestions from Dr. M. Aslam:

Dr. M. Aslam started making suggestions by mentioning, “we can now witness the fruits of our efforts and the progress arising from the seeds we sowed during the early stages of the DBT-NECAB programme”. In continuation, he further said DBT-NECAB has truly set a commendable example of development through conducting training programmes, utilization of resources, and the generation of output in the form of product and technique development. Dr. Aslam expressed his gratitude to Dr. Sarmah and all the members for the remarkable progress they have accomplished.

Dr. Aslam also praised the effort of developing a new variety of rice at DBT-NECAB programme, which was released in June 2023. Dr. Aslam asked to let him know about such important development well in advance so that these varieties can be released virtually by the honorable minister, Science and Technology, GoI.

Dr. Aslam praised the work of all the programme leaders and said everyone is doing exceptionally well. Dr. Aslam also introduced the SAC members to different entrepreneurship programmes funded by DBT, DST, and ICAR. He encouraged all the PIs and young scientists to apply for individual research grants, for example Biotechnology Ignition Grant (BIG) from BIRAC supports with 50 lack rupees for converting idea into proof of concept in a period of 18 months.

Dr. Aslam said that the scientific research and training programmes conducted by the DBT-NECAB programme have elevated the North-East region to a new level. Now this platform is capable of not only mentoring the satellite labs but also other institutions in rest of India.

According to the new financial system, continuous and timely responsiveness is required from both the accounts and administration sections at AAU, Jorhat. Dr. Aslam suggested that the expenses must be strategically managed so that funds allocated for one year are fully utilized within the period of validity, and funds for the next year must be requested without any delay because delaying both expenditure and grant requests can result in financial losses. The reason is that after 31st March (the end of the financial year), the allocated funds will be reflected as zero in the central nodal account. Subsequently, requesting a revival and resetting of the limits may raise concerns with DBT on this matter. So, it is very important to effectively manage funds both in the process of requesting and expenditure.

Dr. Aslam also make everyone aware of the new financial system and said that the new financial system provides flexibility in fund utilization. From the second year, only recurring and non-recurring funds will be received. This new financial will provide more flexibility in fund management and spending. Plans can be made based on actual requirements. For example, there is no need to provide a detailed manpower statement. Instead, one can simply submit a certificate by mentioning that all the manpower positions in the project comply with the DST guidelines. Details such as the name of the person, employment period, amount of payment are not required.

A well-prepared work plan spanning four and a half years should be prepared for Project III, IVa, and IVb. More emphasis should be placed on project III. The work plan should include the basic components, objectives, research methods, bioprospecting genes related to acid tolerance, microbial studies, and other elements utilized in the development of bioformulations.

At the end, Dr. Aslam congratulated and thanked all individuals associated with the DBT-NECAB programme.

Response from Dr. B. K. Sarmah:

Dr. B. K. Sarmah appreciated all the valuable suggestions provided by the SAC members. He requested the committee to convey a message to the DBT and AAU administration regarding the necessity of making some of the positions permanent after the completion of phase III. These

positions are 2-3 scientists, Instrumentation Engineer, Information Technology (IT) consultant, and a bioinformatics expert. Such positions are important for sustaining this Centre of Excellence.

Response of Dr. Aslam:

The phase III programme has just started so it is too early to think of phase IV. Regarding the positions, everything is possible through extramural funding. As per DST guidelines, there are provisions for project scientist and IT expert. It would be good to float the idea of permanent centre during the third meeting of the Phase III programme. However, the recommended proposal for permanent positions should be promptly forwarded from this committee to the AAU administration. Dr. Aslam mentioned that the University authority can smartly absorb these positions as permanent.

Response of Dr. Utpal Bora:

Dr. Utpal Bora also supported Dr. Aslam response and suggested to put the proposal of a permanent institution during the 3rd year of the phase III programme. Dr. Utpal Bora highlighted the importance of establishing arrangements that can ensure the long-term sustainability of this programme because a huge investment has been made in building such an excellent infrastructure. Later, Dr. Utpal Bora proposed considering the establishment of a repository where all the resources such as samples, microbial cultures etc. should be stored. Dr. Sarmah replied that at AAU, there is no such system, but our samples such as transgenic lines are available at the NBAIM national repository.

Dr. N. K. Singh:

Dr. N. K. Singh suggested that DBT-NECAB could think of generating some resources through the licensing of the produced products. Earlier, there was a rule in ICAR that everything you generate you must give back to them but now ICAR has revised its policy and is requesting only 30% of the generated resources.

Dr. Sarmah replied that DBT-NECAB is already generating funds through product sales, certification, and the organization of courses and training programmes. DBT-NECAB is also planning to provide training to undergraduate and master's degree students not only from AAU but also from other North-East institutes in both IT and Bioinformatics.

At the end, Dr. B. K. Sarmah, Director of DBT-NECAB concluded the meeting by extending his appreciation to the SAC members and all faculties of the Dept of Agricultural Biotechnology, AAU; project fellows and office staffs for extending their help for conducting the meeting successfully.