
हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद
Agrisearch with a human touch

# ICAR-IIAB Newsletter

Vol. 1 (1) July- December, 2018

## From Director's Desk

Indian agriculture has made tremendous progress during the last five decades. However, growing population, expanding urbanization and accelerated climate change are creating new challenges for sustainable agricultural growth. In the past, developing world had a challenge to conquer hunger, but now the challenge is to conquer hidden hunger by providing adequately nutritious diet to poor masses. With all these challenges, India has a major task to ensure food and nutritional security to the most populous country by 2050 with one of the largest malnourished population. On the other hand, India is also facing a challenge to retain small-holder farmers in farming as a source of livelihood. Biotechnological interventions can play a key role in addressing these challenges for large as well as small land-holder farmers. Unprecedented advancements in biotechnology, information technology and nanotechnology have already started offering tremendous opportunities for application in various sectors, including agriculture. Realizing tremendous progress in frontier areas of biotechnology and advantages realized by the farmers using biotechnology enabled technologies, ICAR has established ICAR-Indian Institute of Agricultural Biotechnology at Ranchi in 2012. The institute shall cater to future demand of biotechnology products, processes, technologies and world class human resource trained in frontier areas of biotechnology encompassing all spheres of agricultural biotechnology, including plant, animal, fish and microbial biotechnology. The IIAB is envisioned as an independent institute under ICAR with Deemed to be University status. Since its establishment in 2012, the institute is operating with modest research facilities developed with in the limited space at IINRG Campus. However, with kind support from the ICAR headquarters, the approval of master plan was conveyed and funds have been provided for construction of Field Crop Research and Training Centre; Livestock Research and Training Centre; and Fisheries Research and Training Centre in Farm B of IIAB. In addition, the construction work of Administrative & Institute Building, Hostels, Mess Building and Director's Residence is expected to start soon.

Research programs have been developed under three major areas *viz*., Genomics and Bioinformatics; Translational Research for Crop Improvement; and Fish Health Management. The institute has signed MoU with IIT, Guwahati; Birsa Agricultural University, Ranchi and Vinoba Bhave University, Hazaribag for sharing available facilities and human resource development. In addition, the institute is also undertaking skill development and capacity building activities among farmers, entrepreneurs and stakeholders under various programs, such as Farmers FIRST, TSP, FLD, SCSP and MGMG etc.

It gives me immense pleasure to congratulate the entire IIAB family on publication of its first issue of Newsletter, which has showcased the major achievements of scientists and staff and the activities carried out under various programs during July-December, 2018.

### Contents



## Foundation Stone Laying Ceremony

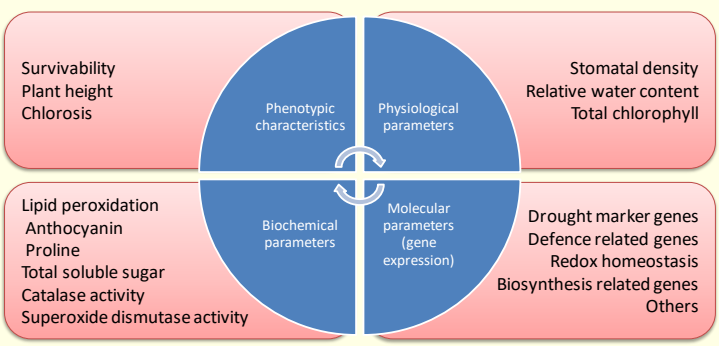
Foundation stone of ICAR-IIAB Field Crop Research and Training Centre was laid by Dr. T Mohapatra, Secretary DARE and Director General, ICAR, New Delhi on Aug. 26, 2018. On this occasion, Dr. Anand Kumar Singh, DDG (CS) ; Dr. DK Yadava, ADG (Seeds), Dr. RK Singh ADG (CC); Dr. TR Sharma, Director, IIAB and Directors of other ICAR Institutes in Ranchi and Vice Chancellor of Birsa Agricultural University, Ranchi were also present. At Farm B of the institute, different units of Field Crop Research and Training Centre, Livestock Research and Training Centre and Fisheries Research and Training Centre along with the developmental works including roads, rain water harvesting ponds etc. will be constructed as per approved master plan.

# Research Highlights

## Screening of lentil (*Lens culinaris*) genotypes for drought tolerance

Ragini Sinha, Awadhesh K. Pal, Anil Kumar Singh

India is third largest lentil producing country, however it's productivity and yield is significantly reduced by drought stress. Thus, twenty-six lentil genotypes were screened for their drought tolerance and susceptibility. Out of these, eight genotypes were sorted on the basis of their survivability, phenotypic characteristics like chlorosis and plant height. After 15d of drought stress, leaf samples from these eight genotypes were harvested and biochemical parameters, like relative water content (RWC), stomatal density, total chlorophyll, total soluble sugar, anthocyanin and proline contents were assayed. Some of the genotypes showed significant changes in stressed samples with respect to control. Following this, the gene expression analysis for drought marker genes like ERD, Dehydrins, DREBs etc. was performed and further three genotypes were selected as putative drought tolerant (GP3643, IC248956) and drought susceptible (GP3690). In order to understand the stress perception and downstream signalling, transcript analysis of various genes related to diverse metabolic and biochemical pathways was also performed. However, screening of these potential drought tolerant and susceptible genotypes at field level may be required to ascertain their relative drought tolerance.




**Workflow of experimental parameters followed for screening drought tolerant lentil**

## Identification of stable reference genes for qRT-PCR data normalization in lentil (*Lens culinaris*)

Ragini Sinha and Anil Kumar Singh

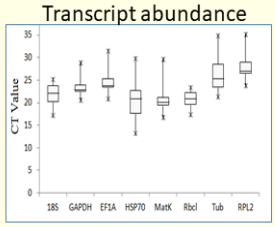
Avaliability of stable reference gene is a prerequisite for qRT-PCR data normalization in an experimental condition. In order to identify stable reference genes in lentil, expression stability of eight candidate genes *viz*. Rbcl, RPL2, 18S rRNA, tubulin, EF1α, GAPDH, HSP70, and mat K was evaluated in five varieties of lentil at three different stages of leaf development and abiotic stress conditions. The results were analysed using geNorm, BestKeeper, NormFinder and RefFinder programs. All programs identified RPL2 as most stable under abiotic stress and developmental stages followed by Tub and Rbcl.

While, HSP70 was identified as least stable. This study may help in selection of appropriate reference gene(s) for qRT-PCR data normalization in lentil depending upon the experiment.

qPCR parameter of primers

| Sl No. | Gene | Cor. Coeff. ($R^2$) | Eff. (%) |
|---|---|---|---|
| 1 | RPL2 | 0.959 | 86.3 |
| 2 | EF1α | 0.999 | 98.5 |
| 3 | HSP70 | 0.999 | 101.3 |
| 4 | Rbcl | 0.986 | 101.7 |
| 5 | Tub | 0.995 | 99.3 |
| 6 | Mat K | 0.99 | 96.9 |
| 7 | 18S | 0.998 | 98.8 |
| 8 | GAPDH | 0.957 | 112.3 |

Transcript abundance




Comprehensive Ranking of genes stability

| Rank | All sample type | | | | Abiotic stress conditions | | | | Developmental stages | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Best Keeper | geNorm | Norm-Finder | Ref-Finder | Best Keeper | geNorm | Norm-Finder | Ref-Finder | Best Keeper | geNorm | Norm-Finder | Ref-Finder |
| 1 | RPL2 | RPL2 | RPL2 | Rbcl | RPL2 | RPL2 | RPL2 | RPL2 | Rbcl | RPL2 | RPL2 | HSP70 |
| 2 | GAPDH | Rbcl | mat K | Mat K | 18SrRNA | Tub | Tub | Rbcl | RPL2 | Mat K | Tub | RPL2 |
| 3 | Rbcl | EF1α | Rbcl | RPL2 | GAPDH | GAPDH | GAPDH | Tub | 18SrRNA | EF1α | 18SrRNA | Rbcl |
| 4 | EF1α | Mat K | 18SrRNA | EF1α | Rbcl | Rbcl | Rbcl | GAPDH | Tub | Tub | Mat K | 18SrRNA |
| 5 | Mat K | GAPDH | Tub | GAPDH | Tub | EF1α | EF1α | 18SrRNA | EF1α | Rbcl | Rbcl | Tub |
| 6 | 18SrRNA | 18SrRNA | GAPDH | Tub | EF1α | 18SrRNA | 18SrRNA | Mat K | HSP70 | GAPDH | HSP70 | Mat K |
| 7 | Tub | Tub | EF1α | 18SrRNA | HSP70 | Mat K | Mat K | EF1α | Mat K | Hsp70 | GAPDH | EF1α |
| 8 | HSP70 | HSP70 | HSP70 | HSP70 | Mat K | HSP70 | HSP70 | HSP70 | GAPDH | 18SrRNA | EF1α | GAPDH |

**Ranking of HKG on the basis of statistical software, followed by data validation through qPCR, using stable (RPL2, Tub) and unstable (HSP70, 18S) genes for normalisation**

## Cloning of heat shock factor (HSF) transcription factor genes from lentil (*Lens culinaris*)

Ragini Sinha, Meenu Bala and Anil Kumar Singh

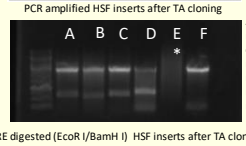
Heat stress is one of the universal stresses faced by all organisms. To cope with heat stress, plants have developed large heat shock factor (HSF) transcription factor families and a complex transcriptional network composed of many transcription factors. Activation of HSFs is an important step for initiation of the heat stress response. Homologous HSF gene sequences were obtained through NCBI and Knowpulse database. The expression of nine HSFs (HSFA3, HSFA8, HSFA4a, HSFA1b, HSFB2, HSFB2a, HSF25, HSF33 and HSF34), was analysed in heat stressed leaf samples of lentil. The genes were amplified from lentil using PCR and cloned in TA cloning vector. These partial fragments were sequenced and found homologous to corresponding HSF isoforms. In order to perform the functional characterisation of HSF genes, full length gene sequences were determined using RACE (Rapid amplification of cDNA ends).

HSF primer sequence

| Gene | Nucleotide Sequence | Tm | Amplicon (bp) |
|---|---|---|---|
| LC_HSFA3 FP: | GCAGGGAAATTCGGAGCCGAA | 59°C | 621 |
| LC_HSFA3 RP: | TTGAAAGCCTTCAAAATTCCCAG | | |
| LC_HSFB2a FP: | TTCCTTCACCTATGCCGCTGA | 57°C | 577 |
| LC_HSFB2a RP: | TAATTCGCTCTACTACACCGG | | |
| LC_HSFA8 FP: | ATCATCACACAAAGCTTCAGATGGA | 61°C | 684 |
| LC_HSFA8 RP: | GCCAAATTGCCAATTTCTTGACAA | | |
| LC_HSFA4a FP: | GGCAACCGCAGCTTATCAAGA | 57°C | 935 |
| LC_HSFA4a RP: | AATTTCTCTACTTGCCAAC | | |
| LC_HSFA1b FP: | AAGGGATAAAAATGTTCTTATGAC | 57°C | 592 |
| LC_HSFA1b RP: | CAACACAGAAGGGGAAGATTG | | |
| LC_HSF33 FP: | AAAGAGACAAGAATGTTCTGATGC | 59°C | 532 |
| LC_HSF33 RP: | CCTGTTGGAGCTGGAATG | | |
| LC_HSF34 FP: | ATGTCGCAGCGGTCGGTG | 55°C | 205 |
| LC_HSF34 RP: | CATATGTGTTGAGCTGGCGAA | | |
| LC_HSF25 FP: | TCGGTGCGTGGGCCCGTTT | 57°C | 193 |
| LC_HSF25 RP: | CATATGTGTTGAGCTGGCGAA | | |
| LC_HSFB2 FP: | TGTGTTACCACTGAGGAGTTG | 57°C | 177 |
| LC_HSFB2 RP: | AATCTTCGTTAAGTACCGC | | |

Gene Expression and TA cloning

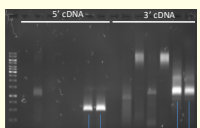

PCR amplified HSF inserts after TA cloning



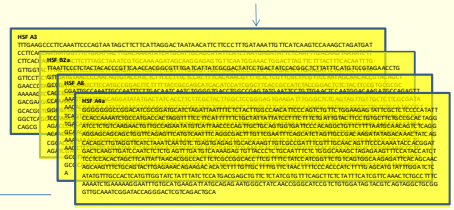

A: Clone HSF A3
B: Clone HSF B2a
C: Clone HSF A8
D: Clone HSF A4a
E: Clone HSF A1b
F: Clone HSF 34
E*: No Fall out

RE digested (EcoR I/BamH I) HSF inserts after TA cloning

Sequencing and BLAST search




Full length Sequencing: RACE



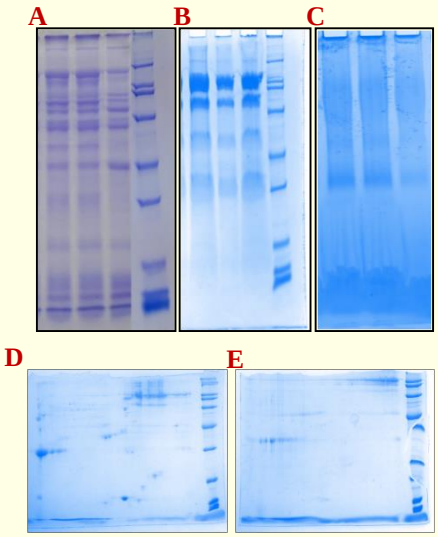
HSF Partial Sequences

**Cloning of heat shock factor (HSF) transcription factor genes from lentil (*Lens culinaris*)**

## Characterization of molecular marker(s) associated with X- and/or Y-chromosome bearing spermatozoa in cattle

**Laxmi Vandana Rongala & Soumen Naskar**

Sperm sexing in cattle offers a favourable breeding strategy for meeting the increased requirement of food production. Currently used methods for sperm sexing suffer from lower accuracy, poor repeatability, and render low fertility. In view of this, research on development of innovative technology with rate and purity of sperm sorting without affecting its viability is crucial. The present study was aimed to identify different plasma membrane proteins associated with X- and/or Y-chromosome bearing spermatozoa of cattle. Preliminarily, total proteins were extracted from unsorted sperm cells and extraction of plasma membrane associated proteins was carried out from the plasma membrane fraction of the unsorted semen that was collected from the post-nuclear supernatant of homogenized sperm cells by high speed centrifugation. Gel electrophoretic profiling of the extracted total soluble proteins and plasma membrane associated proteins from unsorted bovine sperm cells was carried out. Apart from the solubilisation issues, the hydrophobicity of the plasma membrane associated proteins prevented their entry into/movement in the gel during IPG-IEF, SDS-PAGE and blue native PAGE, due to which gel-free approach, in-solution digestion with trypsin and lys-C and LC-MS/MS, was carried out to identify the proteins. To summarize, a novel extraction method has been developed for enrichment of plasma membrane proteins from bovine sperm cells.

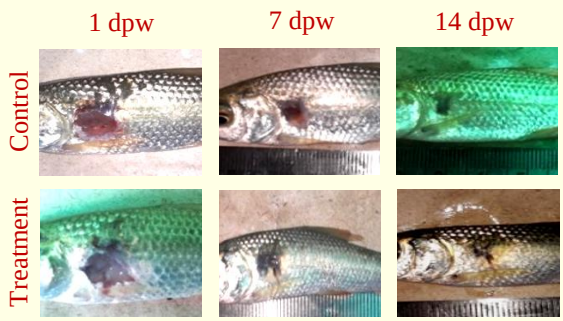



**Gel electrophoretic profiling of extracted total soluble (TS) proteins and plasma membrane (PM)-associated proteins from unsorted bovine sperm cells. 1DGE/SDS-PAGE of (A) TS proteins, and (B) PM-associated proteins; (C) Bis Tris Blue Native PAGE of PM-associated proteins; 2DGE of (D) TS proteins and (E) PM-associated proteins.**

## Impact of nanosilver- composite on wound healing property of *Labeo rohita*

**Biplab Sarkar & Sanjay Kumar Gupta**

The effect of silver nanoparticles (Ag-NPs) composite on wound healing efficiency of *Labeo rohita* was investigated. Sixty fingerlings of *L. rohita* (av. Weight: 8.04± 0.32 g) were randomly distributed into two treatment groups *viz*. control and treated with 3 replicates of each. Prior to commencement of trail, incisional wound was created by sterilized blade posterior to the base of pectoral fin. Nanosilver- composite were directly applied to the wounded site. Wound size was found to be gradually decreased in both the groups during 14 days healing period, however significantly higher reduction in wound size was observed in Ag-NPs- composite group, compared to control. The preliminary results indicate that, Ag-NPs- composite is effective in gradual healing of wound. Hence Ag-NPs composite could be used for wound healing treatment on fish in order to improve aquaculture production.

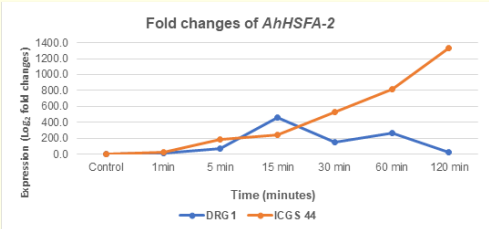



**Impact of nanosilver-composite on wound healing of *Labeo rohita* fingerlings after 1, 7 and 14 days post-wound (dpw)**

## Possible role of AhHSFA-2 in heat stress tolerance in groundnut

**Bhubaneswar Pradhan & Sujit Kumar Bishi**

Groundnut crop optimally grows up to 35°C and its growth gets hampered beyond 40°C. Hence, cultivars with enhanced heat stress tolerance are required to minimize yield loss. Groundnut genotypes differing in heat stress tolerance *viz*. DRG 1 (susceptible) and ICGS 44 (tolerant) are available, but the underlying molecular mechanism is not known. Plants circumvent heat stress by expressing heat-shock proteins (HSPs), which is controlled by heat-shock factors (HSFs). We characterized a putative HSFA-2 in groundnut. A time-course induction pattern of *AhHsfA-2* was studied by exposing 7 d old seedlings to heat stress (42°C). Expression analysis using qRT-PCR showed that *AhHsfA-2* is induced within a min after heat stress treatment, in both the genotypes. Its expression reached peak (465 fold) within 15 min and substantially reduced within 120 min (25 fold) in DRG 1. While, in ICGS 44, its expression was induced continuously (1329 fold) until 120 min. Interestingly, the tolerant genotype, ICGS 44 maintained a higher induction of *AhHsfA-2* than that of the susceptible genotype, suggesting its possible role in heat stress tolerance in groundnut.




**Expression profile of *AhHsfA-2* in DRG 1 and ICGS 44 cultivars of groundnut**

## Characterization of genes responsible for immune response in *Labeo rohita* fingerlings post dietary feeding with microbial levan

**Sanjay Kumar Gupta, Soumen Naskar, Biplab Sarkar**

The dietary administration of microbial levan at 1.25% in *A. hydrophila* infected *L. rohita* fingerlings has shown the significant upregulation of m-RNA mediated pro-inflammatory cytokines IL-1β, TNF-α and IL-12p40; and downregulation of anti-inflammation regulatory cytokine IL-10 in the intestine, gill, kidney and liver. Furthermore, increased respiratory burst activity and serum lysozyme activity were observed in a time dependent manner which confirms the immune-stimulatory effect of levan on *L. rohita*.

# Meetings and Events

### Research Advisory Committee (RAC) Meeting

The 6th RAC meeting of ICAR-IIAB was organized on Nov. 29-30, 2018 . The RAC comprised of Padma Bhushan Prof. VL Chopra, Chairman, former Director General, ICAR and former Member Planning Commission; Prof. HS Dhaliwal, Vice Chancellor, Eternal University, Sirmour, HP; Prof. K Veluthambi, former Head, Dept. of Plant Biotechnology, Madurai Kamraj University; Dr WS Lakra, former Director, ICAR-CIFE, Mumbai; Prof. KR Koundal, former Joint Director (R), IARI, New Delhi & Scientist Emeritus, NRCPB, New Delhi; and Dr. BP Mishra, Joint Director (R), IVRI, Bareilly; Dr. TR Sharma, Director, ICAR-IIAB and Dr. VP Bhadana, ICAR -IIAB. Dr. TR Sharma welcomed chairman and members of RAC and presented progress of infrastructure and research facilities development at IIAB. Action taken report on recommendations of 5th RAC meeting was presented by Dr. VP Bhadana. Head In-charge of different schools, Dr. VP Bhadana, Dr. Biplab Sarkar and Dr. Anil K Singh presented progress made under respective thematic areas in different research projects. Chairman and members of RAC expressed satisfaction on substantial progress in research on the relevant regional research gaps and thrust areas despite daunting constraints. Dr. TR Sharma, Director expressed his gratitude to the Chairman and members of RAC for their valuable suggestions.


### Institute Management Committee (IMC) Meeting

The 3rd meeting of Institute Management Committee (IMC) was held on May 30, 2018. Dr. TR Sharma, Chairman IMC & Director, ICAR-IIAB, Ranchi; and members of IMC, Dr. DK Yadava, ADG (Seeds), ICAR, New Delhi; Dr. JC Rana, National Coordinator, United Nations Environment GEF Project, Bioversity International, Dr. Anil Rai, Head, Centre for Agricultural Bioinformatics, ICAR-IASRI, New Delhi; Dr. Vindhya Mohindra, Head, Fish Conservation Division, NBFGR, Lucknow; Dr. Kishor, Gaikwad, Principal Scientist, ICAR-NRCPB, New Delhi; Shri Kameshwar Oraon, AAO, ICAR-IIAB, Ranchi attended the meeting. Dr. VP Bhadana, PS, IIAB; Dr. Biplab Sarkar, Sr. Scientist, IIAB; Dr. Anil K Singh, Sr. Scientist, IIAB; Dr. Faheem Ansari, Sr. Scientist and Incharge, Finance and Accounts, IINRG also attended the meeting as invitee members. The IMC discussed about various management issues related to the institute.

## Institute Research Council (lRC) Meeting

The meeting of Institute Research Council (lRC) was held on August 18, 2018. The meeting was chaired by Dr. T. R. Sharma, Director. He emphasized that scientists should focus more on the local problems followed by regional problems, *i.e.* Eastern India in general and Jharkhand in specific in view of the envisaged Second Green Revolution and doubling of farmers' income. He stressed on development of interdisciplinary project proposals encompassing all three commodities, *viz.* Crop, Animal and Fish, to showcase the uniqueness of the Institute. He also stressed on surveying farmers' field for problem identification and analyze the research/knowledge gap. Based on the recommendation of the RAC, formation of the third school of the institute, *i.e.* "School of Genetic Engineering" was approved. Review of the ongoing institute and externally-funded projects was done.

## Vigilance Awareness Week

Vigilance Awareness Week' was celebrated with grandeur at ICAR-IIAB, Ranchi, Jharkhand during 29th October to 3rd November, 2018. On 29th October, the Awareness Week was initiated with 'oath taking ceremony' by all staffs. One 'elocution competition' was organized on the given thematic area, 'Eradicate Corruption- Build a New India' participated by all staff members of the institute. Best speakers were given prizes which were decided by panel members. The valedictory cum sensitization programmes were organized on 3rd November from 2.30 PM onwards. In this session, Mr. Sanjay Agarwal, DGM and Additional Chief Vigilance Officer SAIL-Research & Development, Ranchi, Jharkhand was invited as Chief-Guest of the programme and he delivered a talk on important rules of vigilance practiced in central government institutes. Dr. V.P. Bhadana, Principal Scientist, ICAR-IIAB mentioned about the importance of vigilance in the ICAR system. The programme concluded with comments from Director, followed by vote of thanks from Dr. Biplab Sarkar, Senior Scientist and Vigilance Officer, ICAR-IIAB.

## हिन्दी चेतना मास

राजभाषा अधिनियम के अनुपालन एवं कार्यालय कार्य में हिन्दी के प्रयोग में उत्तरोत्तर वृद्धि के लिए संस्थान में दिनांक-01.09.2018 से 30.09.2018 तक हिन्दी चेतना मास का पालन किया गया। इसके अन्तर्गत दिनांक-29.09.2018 को अपराह्न 02.15 बजे हिन्दी दिवस समारोह का आयोजन किया गया। हिन्दी दिवस समारोह के अवसर पर मुख्य अतिथि के रूप में श्री अमरकान्त, प्रबंध संपादक, खबर मंत्र, हिन्दी दैनिक, राँची तथा डॉ हीरानन्दन प्रसाद, साहित्यकार एवं सह प्राध्यापक, हिन्दी विभाग, राँची विष्वविद्यालय विशिष्ट अतिथि के रूप में उपस्थित थे। श्री अमरकान्त ने संस्थान के शोध और उपलब्धियों को हिन्दी में निकालने की बात कही, जिससे स्थानीय स्तर पर किसान और ग्रामीण उसे पढ़कर लाभान्वित हो सकें। उन्होंने कहा कि यह काफी सराहनीय बात है कि संस्थान के वैज्ञानिक बातचीत और अपने कागजाती कार्य में हिन्दी का ही प्रयोग करते हैं। विशिष्ट अतिथि डॉ हीरानन्दन प्रसाद ने अपने संबोधन में कहा कि मेडिकल साईंस और अन्य टेक्नोलॉजी की पढ़ाई के लिए अंग्रेजी में लिखी किताबों का हिन्दी में अनुवाद होना चाहिए, तभी छात्र-छात्राओं का रूझान इस ओर बढ़ेगा। संस्थान के निदेशक, डॉ तिलक राज शर्मा ने हिन्दी दिवस समारोह के अवसर पर शुभकामनाएं दीं तथा संस्थान में हिन्दी के अधिक से अधिक प्रयोग संबंधी प्रयासों के बारे में बताया। कार्यक्रम का संचालन डॉ अन्जेष कुमार, वरिष्ठ तकनीकी अधिकारी ने किया।

### Activities under Swachh Bharat Mission

Under Swachh Bharat Mission, ICAR-IIAB organized fortnight long programs during Sep. 15-Oct. 2, 2018 under Swachhata hi Seva campaign and cleanliness drives during Dec. 16-31, 2018. Swachhata pledge was taken by all the staff members. The Swachhata hi Seva program was inaugurated by Shri Chhabilendra Roul, Special Secretary, DARE & Secretary ICAR by planting trees at IIAB, Garhkhatanga campus. Cleaning work was done in institute campus and nearby school, public/tourist places. Awareness on importance of cleanliness, hygiene and waste disposal etc. was imparted through various programs in nearby villages and schools.




### Participation in ICAR-Zonal Sports Tournament for Eastern Zone

A 10-member sports contingent of ICAR-IIAB, Ranchi participated in the ICAR-zonal sports tournament for eastern zone hosted by ICAR-IINRG, Ranchi during 5$^{th}$ -8$^{th}$ October, 2018. The team ICAR-IIAB participated in the tournament with the coordination of Dr. Madan Kumar as Chief-De-Mission and Dr. Biplab Sarkar as Team Manager. The zonal sports tournament was attended by 502 participants from 18 ICAR institutes located in 12 states of Eastern zone. The Director congratulated the team ICAR-IIAB for their sportsmanship spirit shown in the zonal sports tournament.

## *Institutional Activities*

### Activities under Tribal Sub Plan (TSP)

Under the Tribal Sub Plan (TSP), ICAR-IIAB supported a total of 1,008 tribal farm families from 10 villages of three districts of Jharkhand namely *Latehar*, Hazaribag, and Ranchi. Improved production technologies for field crop, horticulture, agroforestry, fishery, backyard poultry and critical agricultural inputs were provided to the tribal farmers. The interventions taken up were (1) promotion of cultivation of drought-tolerant high yielding rice variety DRR - Dhan 42 along with other high yielding rice varieties, namely Sahabhagi Dhan, Abhishek and Lalat; (2) promotion of cultivation of high yielding Indian mustard varieties namely NRCHB-101, PM-26 and PM-27 in the rice fallows for increasing cropping intensity and farm income; (3) promotion of cultivation of papaya; (4) promotion and popularization of improved germplasm of fish and poultry, and (5) promotion of cultivation of mahogany, a fast growing high value timber tree. The critical inputs like seeds, fertilizers, insecticides, fungicides, antibiotics, pheromone traps etc. for taking up 100 hectares each of rice and mustard cultivation; seedlings, fertilizers, anti-termite chemicals etc. for taking up high intensity papaya cultivation in 10-12 hectares; 2,000 saplings of mahogany; 25,000 fish fingerlings for homestead ponds were provided to the tribal farmers. Moreover, a series of farmer-scientist interaction meetings, on-farm training, field days, on-farm trials, farmer's fair and outstation exposure visits etc. were conducted. Two short term (4-5 days) trainings on mustard cultivation and Pisciculture at ICAR-DRMR, Bharatpur and Fish Farm Training Centre, Ranchi, respectively, were organized for the tribal farmers. Specialized training on Farmer Producer Organization (FPO) was conducted to induce a sense of entrepreneurship among tribal farmers. On farm trial of Divyayan Red and Khaki Campbell birds at *Lalkhatanga*, *Garhkhatanga*, *Kharsidag* and *Kochbang* villages under Namkum block of Ranchi, was conducted. Divyayan Red chicks, Khaki Campbell ducklings and compound feed were provided to the stakeholders. The birds were vaccinated against prevalent diseases before distribution. Orientation programme on scientific management of backyard poultry was organized. A multidisciplinary team of scientists from ICAR-IIAB, Ranchi regularly monitored the implementation of programme. Dr. T.R. Sharma, Director, ICAR-IIAB, Ranchi also visited some of the locations and expressed satisfaction over the efforts made by the TSP implementation team.



## Activities under Front Line Demonstration (FLD)

During the year 2018-19, through Front Line Demonstration (FLD) Programme, ICAR-IIAB demonstrated a cafeteria of rice technologies in 30 hectares of rainfed upland area of 54 farmers from 14 villages of three districts of Jharkhand namely *Latehar*, Hazaribag, and Ranchi. FLDs organized during the year have been effective in creating awareness about the potential of drought-tolerant rice variety DRR-Dhan 42 under drought conditions. Since protein-energy malnutrition is common among the tribal children of Jharkhand, ICAR-IIAB also conducted a significant number of FLDs of two newly released high-protein rice varieties namely CR Dhan 310 and CR Dhan 311. Although some of the tribal farmers seemed to be skeptical about these varieties, a recurrent motivation by the ICAR-IIAB scientists through a series of farmer-scientist interaction meetings, training, field days, etc. lead to general acceptance and interest in the varieties by the farmers. The FLD programme of ICAR-IIAB has been successful in convincing the farmers about the effectiveness of scientific crop management practices. The technologies demonstrated through FLDs recorded the average yield advantage of 11.8% over the farmers' practice. The FLDs revealed that there is tremendous scope to bridge the yield gaps in rainfed uplands of Jharkhand by large scale adoption of drought-tolerant high yielding rice varieties.


## Activities under Mera Gaon Mera Gaurav (MGMG)

Under *Mera Gaon Mera Gaurav* programme, village namely *Chetag* under *Balumath* block of *Latehar* has been selected to provide the required knowledge and regular advisory. A multi-disciplinary team of scientists from ICAR-IIAB, Ranchi made visits during 2018 and interacted with the farmers of village for identifying the significant problems and provided ample solutions to the farmers.

# Infrastructure Development

As per approved Master Plan of ICAR-IIAB, construction of Farm Office-cum-Field Lab, Godown and Farm Implement Shed & Thrashing Yard of Field Crop Research and Training Centre, Farm Office-cum-Training Hall of Livestock Research and Training Centre and Fish Wet Labs and ponds of Fisheries Research and Training Centre along with the developmental works including roads, rain water harvesting channels and water storage pond is under progress.

*Biotechnology Update*

# Genome editing using CRISPR-Cas9: another band wagon or an essential tool for genetic improvement?

**Sujatha TP, Md. Imran Ahmad, BK Singh, Madan Kumar, Rishikesh Kumar, Sudhir Kumar, Avinash Pandey, VP Bhadana, R Srinivasan, TR Sharma**

Genome editing is a set of tools or techniques, to change the genetic information of an organism by adding, deleting, altering or replacing fragments of DNA in specific, targeted loci in the genome. CRISPR-Cas9 system is most important, simple, versatile, more precise, efficient, predictable, faster and cheaper, genome editing tool than other methods. CRISPR-Cas9 was discovered first in *Escherichia coli* in 1987 and unveiled by Science magazine as "Breakthrough of the year" in 2015. It is a prokaryotic adaptive immunity system in bacteria and archaea, to eliminate invading viruses. A customisable, synthetic, nearly 20 bases long single guide-RNA (gRNA) directs Cas9 endonuclease to specific targets in the genome to create double strand breaks, which are repaired through non-homologous end joining (NHEJ) and homology directed rapair (HDR).

Unlike genetic engineering, genome editing is not random and does not involve gene transfer from other organisms. On-target efficacy of the RNAi and CRISPR-Cas technologies are similar, though off-target effects of RNAi are much greater. Crops undergoing siRNA-mediated gene silencing had safety concerns. Also, elucidating the role of each gene family member is not possible with RNAi. CRISPR-Cas9 is more popular and may replace RNAi in the long term.

CRISPR-Cas9 technology has been successfully employed to decode functions of genes, incorporate traits for biotic and abiotic stress tolerance, male sterility, nutritional qualities, agronomic traits and to modulate the transcriptome and epigenetic mechanisms in plants (Table).

| Sl.No | Applications | Target genes and effects |
|---|---|---|
| 1 | Decode functions of genes | PHOSPHATE1 (PHO1) in Pi transport in tomatoes (Zhao et al. 2018) |
| | | BIG gene in auxin transport and seeding viability of rice (Cheng et al. 2019) |
| 2 | Tolerance to biotic stress | eIF4E and eIF4G the host susceptibility factors for viral translation in for viral resistance in *Cucumis sativus* and rice, respectively (Chandrasekharan et al., 2016; Macovei et al., 2018)<br>TaMLO-A1 and SlMlo1, host susceptibility genes for powdery mildew resistance of wheat and tomato, respectively (Wang et al. 2014; Nekrasov et al. 2017)<br>OsERF922, a transcription factor involved in multiple stress responses for blast resistance in rice (Wang et al. 2016).<br>SWEET13, a sucrose transporter for bacterial blight resistance of rice (Zhou et al. 2015)<br>Citrus canker susceptibility gene, CsLOB1 edited in Duncan grapefruit ( Jia et al. 2016) |
| 3 | Tolerance to abiotic stress | Editing OsOTS1 class of SUMO proteases in rice for drought and salt stress tolerance (Sadanandom et al. 2019) |
| 4. | Induction of male sterility | P450 fertility gene (Ms26) in maize and its orthologs in rice, sorghum and wheat (Cigan et al. 2016) |
| 5 | Herbicide resistance | Acetolactate synthase (ALS1 and ALS2) for herbicide (chlorsulfuron)-resistance in maize and soybean (Li et al. 2015; Svitashev et al. 2015); EPSPS for glyphosate resistance in rice (Li et al. 2016) |
| 6 | Nutritional quality | Glutinous CMS with low amylose content by knocking out the granule-bound starch synthase OsWaxy and mutations in Waxy (Wx) locus encoding GBSSI in rice (Wang et al. 2017) |
| | | GBSS (granule-bound starch synthase) gene-knockout in tetraploid potato (Andersson et al. 2017) |
| | | Modify or delete wheat gliadin genes to remove the immunogenic epitope (Sanchez-Leon et al., 2018) |
| | | Modify or delete ripening-related genes of tomato, encoding the pectin degrading enzymes pectate lyase (PL), led to firmer fruits, while mutations in polygalacturonase 2a (PG2a) and β-galactanase (TBG4) affected fruit colour (Wang et al., 2019) |
| | | Phytoene desaturase (PDS) gene in Cavendish banana (Naim et al. 2018) |
| 7 | Genome imaging | CRISPR-Sirius with enhanced RNA stability and brightness for detection of gene locations (Ma et al. 2018) |
| 8 | Detection of mutations | CRISPR powered e-Transistors (Hajian et al. 2019) |

However, non-specific editing, expression of otherwise dormant genes and unpredictable errors are some drawbacks. Editing may also fail when the repair mechanism uses other chromosome as a template, instead of the newly introduced DNA. In polyploids, verification of editing in all alleles is required. To address these drawbacks, improvement in CRISPR-Cas9 such as use of efficient, high-fidelity variants of Cas9 from *S. aureus,* cytidine base editors (CBEs), for cytidine to thymine base conversion, a programmable ProCas9 for shutting down the CRISPR system, more efficient Cas12a or Cpf1 to produce larger insertions and deletions, Cas12 effectors with range of activities, including target and collateral cleavage of single-stranded RNA and DNA, Cas12b, with small size and high target specificity etc. are made. Online tools and resources, such as, CRISPRPLANT, CRISPR-P 2.0, Cas database, CRISPR-GE, CrisprGE, Cpf1-database and Plant Genome Editing Database (PGED) provide information on tools for designing gRNAs and CRISPR-generated mutants.

The US Department of Agriculture (USDA) does not regulate genome edited plants. In India, genome editing is governed by rules under Environment (Protection) Act,1986 and Rules 1989, similar to that for Genetically Modified Organisms (GMOs). The level of risk associated with genome editing is considered less. Guidelines are released and updated to cover the new technologies, posing risk. Commercial applications and public acceptance are most important for reaping the benefits of nascent CRISPR-Cas9 technology, with immense potential and prospects. CRISPR-Cas has revolutionised molecular biology, but the future challenges will be related to policy and regulation, than scientific or technological concerns.

## Human Resource Development

- Dr. Sujatha, TP attended training programme on "Analysis of Experimental data" at ICAR-NAARM, Hyderabad, Sep. 6-11, 2018.
- Dr. Madan Kumar attended ICAR sponsored short course on Phenomics, the Next Generation Phenotyping (NGP) for Trait Dissection and Crop Improvement at ICAR-IARI, New Delhi, Oct. 20- Nov. 1, 2018.

## Participation in Conferences/Symposium/Seminars/Workshops/ Important meetings

- **Jun. 26-30, 2018:** Dr. Anil K Singh delivered invited talk in 9th International Rosaceae Genomics Conference at Nanjing, China.
- **Jul. 19-20, 2018:** Dr. Anil K Singh delivered invited talk in International Conference on Plant Genetics and Genomics: Next Gen Crops for Sustainable Agriculture held at Chandigarh.
- **Aug. 11-12, 2018:** Drs. Madan Kumar, Sudhir Kumar, Sujatha TP, SK Bishi & Rishikesh Kumar attended 2nd National Conference on Doubling Farmers Income for Sustainable and Harmonious Agriculture DISHA at ICAR-IINRG, Ranchi.
- **Sep. 29, 2018:** Dr. Anil K Singh attended meeting of editors of Indian Journal of Plant Physiology held at Division of Plant Physiology, ICAR-IARI, New Delhi.
- **Oct. 23-26, 2018:** Dr. Anil K Singh delivered invited talk in 6th Plant Dormancy Symposium held at Kyoto, Japan.
- **Oct. 26, 2018:** Dr. Anil K Singh visited Kazusa DNA Research Institute, Chiba, Japan and delivered invited talk.
- **Nov. 22, 2018:** Dr. Sujatha TP attended Orientation Programme of Rashtriya Krishi Vikas Yojana - Remunerative Approaches for Agriculture and Allied Sector Rejuvenation at Krishi Bhawan, New Delhi.
- **Nov. 29-30, 2018:** Drs. TR Sharma & Anil K. Singh attended Global Agriculture and Food Summit 2018 organized by Department of Agriculture, Animal Husbandry and Co-operative, Govt. of Jharkhand.
- **Dec. 2-5, 2018:** Dr. Anil K Singh delivered invited talk in 4th International Plant Physiology Congress 2018 held at CSIR-National Botanical Research Institute, Lucknow.
- **Dec. 6, 2018:** Drs. Soumen Naskar and Sujatha TP attended one-day workshop on "Utilization of Plant Genetic Resources towards Doubling Farmers Income" held at ICAR- RCER, RC, Ranchi.
- **Dec. 7-8, 2018:** Dr. Anil K Singh delivered invited talk in 4th Worldwide Universities Network (WUN) workshop on "Climate Resilient Open Partnership for Food Security" held at Jawaharlal Nehru University, New Delhi.
- **Dec. 14-16, 2018:** Dr. Sujatha TP participated and presented a poster in 1st National Genetics Congress held at Indian Agricultural Research Institute, New Delhi.

## New Externally Funded Projects Launched

- A Department of Science & Technology (DST), Govt. of India sponsored project under WoS-A Programme has been awarded to Dr. Ragini Sinha as PI and Dr. Anil K Singh as mentor with total budget outlay of Rs. 31.75 lakhs.
- A Department of Biotechnology (DBT), Govt. of India sponsored research project under BioCARe Programme has been awarded to Dr. Ragini Sinha as PI and Dr. Anil K Singh as mentor with total budget outlay of Rs. 57.2 lakhs.

## Foreign Deputation

- Dr. Anil K Singh visited Nanjing, China for delivering invited talk in 9th International Rosaceae Genomics Conference during June. 26-30, 2018.
- Dr. Sanjay K Gupta completed 6 months (Jun.- Dec., 2018) Endeavor Research Fellowship at Curtin University, Western Australia.
- Dr. Anil K Singh visited Kyoto, Japan for delivering invited talk in 6th Plant Dormancy Symposium during Oct. 23-26, 2018.

## Awards and Honours

- Dr Anil K Singh, joined as Academic Editor, PLoS One and as Editor, "Indian Journal of Plant Physiology.
- Dr. Sanjay K Gupta, awarded prestigious Endeavour Postdoctoral Research Fellowships by Govt. of Australia.
- Dr. Anil K Singh, appointed as Guest Editor for special issue on "Plant Dormancy" in journal "Tree Physiology".
- Dr. Anil K Singh, appointed as Handling Editor for special section on "Future Crops" in journal "PLoS ONE".
- Dr. Anil K Singh, awarded financial assistance by Indian National Science Academy (INSA), New Delhi for participation in 9th International Rosaceae Genomics Conference held Nanjing Agriculture University, Nanjing, China, during Jun. 26-30.
- Drs. Sujatha TP, Madan Kumar, Sudhir Kumar & SK Bishi, received Young Scientist Award and Dr. Rishikesh Kumar received best Oral presentation award at 2nd National Conference on Doubling Farmers Income for Sustainable and Harmonious Agriculture DISHA, during Aug. 11-12, 2018 at ICAR-IINRG, Ranchi.
- Dr. Anil K Singh, awarded International Travel Support by Science and Engineering Research Board (SERB), Dept. of Science and Technology, New Delhi, Govt. of India for participation in 6th Plant Dormancy Symposium organized by Kyoto University, Kyoto, japan during Oct. 23-26, 2018.
- Dr. Anil K Singh, appointed as eexternal reviewer for project proposals submitted under Scheme for "Promotion of Academic and Research Collaboration" (SPARC) to Ministry of Human Resource Development, Govt. of India.
- Dr. Soumen Naskar, appointed as officer in-charge for Krishi Kalyan Abhiyan Phase I (Jun. 1-Aug. 15, 2018) and Phase II (Oct. 2-Dec. 25, 2018), a programme by the Ministry of Agriculture & Farmers Welfare, Govt. of India, for East Singhbhum district of Jharkhand.

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## Research Publications


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