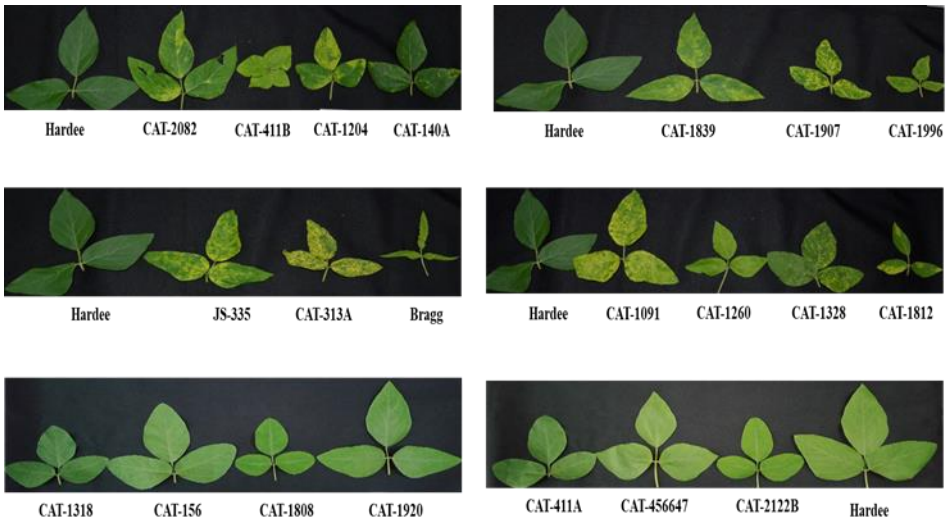
Significant Achievements (SCRSR)

✓ **Screening of minicore accessions of soybean for differential reaction to yellow mosaic disease**

On the basis of field screening of mini-core germplasm subset during kharif of 2020, 2021, 2022, the resistance and susceptible lines were identified based on guidelines by AICRP. These were further subjected to artificial inoculation using viruliferous whitefly under controlled condition. Soybean lines viz., CAT-1809, CAT-1921A, CAT-411B, UPSM-57, and CAT-313A showed highly susceptible reaction while CAT-1318, CAT156, CAT-1808, CAT-411B and EC-456647 were highly resistant to yellow mosaic disease. Other lines showed medium resistance to medium susceptible reaction to YMD



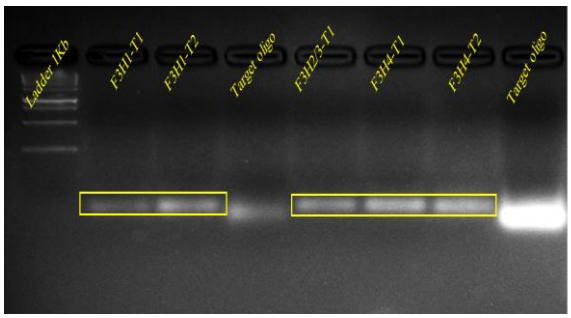
✓ **In-vivo activity of flavonoids against ToLCKV through seed priming**

Total flavonoids from soybean seeds were extracted using standard procedure. Seeds of *Nicotiana benthamiana* were primed with the crude extract for 12 h and subsequently sown with control seeds primed with water. Plants were then agroinoculated with infectious clones of *ToLCKV* at 4 leaf stage for symptom development. Leaves were then harvested (15 days post inoculation) at stage of symptom development for DNA isolation from primed and unprimed *N. benthamiana* plants. To quantify the amount of *ToLCKV* in leaf, the total DNA was subjected to qPCR analysis using *ToLCKV* specific primers. Standard curve of various dilutions of genomic DNA of *ToLCKV* was made based on Ct values obtained from Real time PCR data. Simultaneously PCR was set for the genomic DNA isolated from primed and unprimed tobacco leaves using ToLCKV specific primers. Absolute quantification of viral load was calculated based on Ct values of genomic DNA. The absolute quantification of *ToLCKV* showed that the amount of *ToLCKV* was 28 times lesser in *N. benthamiana* primed with flavonoids extracted from soybean compared to unprimed seeds.

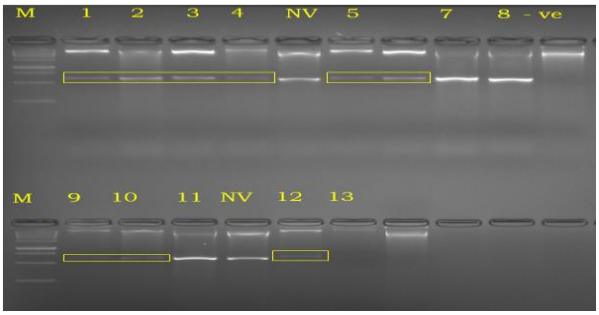
✓ **Designing CRISPR constructs specific to Flavonone-3-hydroxylase gene in soybean**

Target oligos specific to F3H gene in soybean were annealed following the standard procedure cloned into pBLUgRNA vector and resolved on 3% agarose. The annealed oligos were subsequently cloned into pBlugRNA vector. *Escherichia coli* DH5α was transformed with the

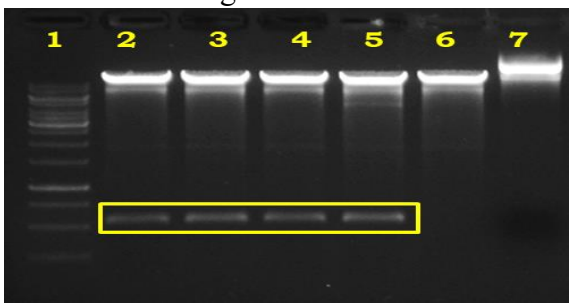
recombinant vector and colonies were screened for integration of target oligos through restriction digestion using EcoRI. gRNA cassette from the positive transformed colonies was then cloned into destination vector pMDC123 containing the Cas9 gene and confirmed through restriction digestion using EcoRI. EHA105 strain of *Agrobacterium tumefaciens* was transformed with the plasmid isolated from positive clones and confirmed through PCR using gRNA specific primers



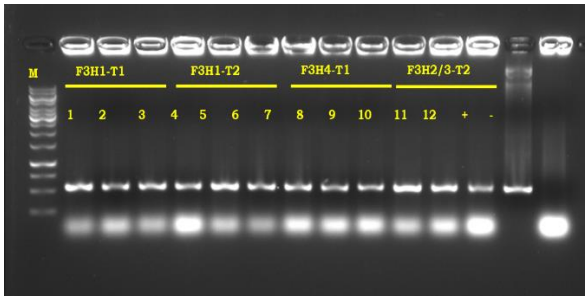
Annealed products analyzed on 3% agarose gel



Restriction digestion of putative transformed colonies



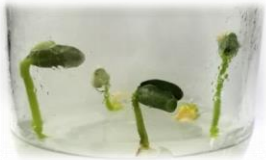
Restriction digestion of clones with EcoRI



Transformation of EHA105 with recombinant Constructs

✓ **Agrobacterium mediated genetic transformation of soybean**

In order to edit two key g e n e s (*Flavonone - 3 hydroxylase* and *Flavone synthase*) involved in flavonoid synthesis, soybean was transformed with recombinant constructs containing the gRNA scaffold and Cas9 gene, through cotyledonary node method. T_0 plants were regenerated on modified medium containing IBA, GA3 and D, L- Phosphinothricin



Germination medium (1/2 B5)

Co-cultivation medium (1/10 B5)

Shoot Induction medium (Day -0)
Full B5 supplemented with BAP (1.6mg/l), cefotaxime (100mg/l) and Amoxycillin (70mg/l)

Shoot Induction medium (Day-10)
Full B5 supplemented with BAP (1.6mg/l), cefotaxime (100mg/l) and Amoxycillin (70mg/l)

Shoot Elongation
Full MS supplemented with GA3 (0.5 mg/l), IBA (0.1 mg/l) cefotaxime (100mg/l), Amoxycillin (70mg/l) and DL-Phosphinothricin (6mg/l)

Hardening of putative transformants in pot culture (Cocopeat: Soil: Sand)

✓ **Improved agroinoculation method for begomovirus infection in legumes**

Stepwise procedure of agroinoculation method by detached one cotyledon of sprouted seeds using dimer constructs of MYMIV DNA A and DNA B (a) germinated seeds after incubation for 24 h (b) germinated seed after removal of seed coat (c) detachment of one cotyledon from the seed coat removed germinated seeds (d) pin-pricking at the site of epicotyl (e) incubation of pin-pricked with agroculture containing MYMIV (both DNA A and DNA B) at 180 rpm, 28 °C for 2 h (f) sowing and growing of agroinoculated seedlings (g) development of yellow mosaic symptoms.

ICAR-CS-NIBSM-Technology-2023-015

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INDIAN COUNCIL OF AGRICULTURAL RESEARCH

Certified that

Dr. P.N. Sivalingam
(Lead Developer)

Associate Developers
Dr. Ashish Marathe
Dr. Pankaj Kaushal
Dr. P.K. Ghosh

of

ICAR-National Institute of Biotic Stress Management
Raipur

has developed the technology

Improved agroinoculation method for begomovirus infection in legumes

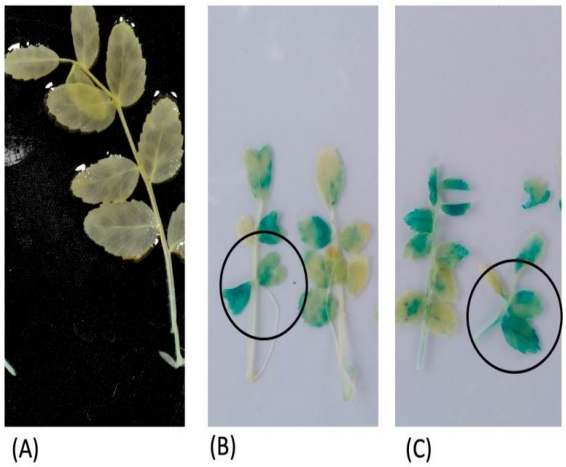
16th July, 2023
New Delhi

(R.K. Singh)
Assistant Director General (CC)

(Tilak Raj Sharma)
Deputy Director General (Crop Science)

✓ Gene specific primers were designed and synthesized for regulatory elements, TFs and R genes, and signaling molecules and used for differential expression using qRT-PCR.

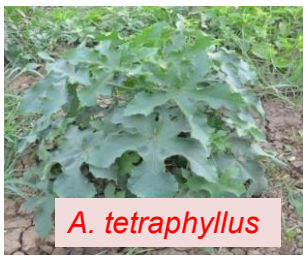
- Role of promoter of IFS1 gene of chickpea against *Fusarium oxysporum* and *Sclerotium rolfsii*

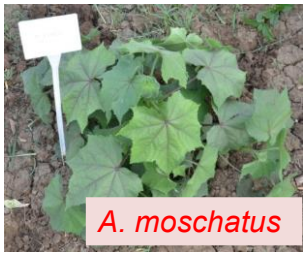


A. Control plants
B. Higher expression of GUS reporter genes observed in leaf samples collected 36 hrs post inoculation using *Fusarium oxysporum*.
C. Higher expression of GUS reporter genes observed in leaf samples collected 36 hrs post inoculation using *Sclerotium rolfsii*

✓ **Identification of novel resistance against YVMV in okra germplasm**

Okra leafhopper resistant lines



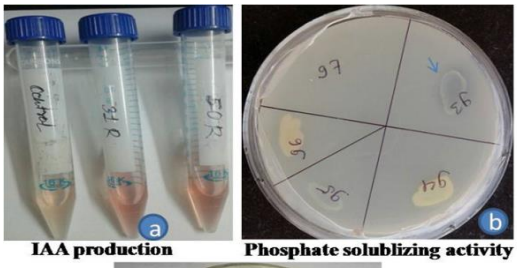


Okra reaction against YVMD

Okra reaction against ELCV

✓ **Identified bacterial endophytes having antagonistic against collar rot disease pathogen (*Sclerotium rolfsii*) and PGPR in chickpea:**

Bacterial endophytes have been isolated from pigeon pea and chickpea plants and theses isolates were tested for their antagonistic activities against soil borne fungal pathogens and PGPR activities. Isolate p53 have shown the ability to suppress collar rot in chickpea and influence growth promotion activities. Bacterial endophyte which have plant growth promotion activities such as root elongation was tested to moisture stress parameter on chickpea plants and showed enhanced tolerance in comparison to the without endophyte bio-primed (Control) plants, these isolates may be explored for studies on abiotic stress tolerance. To identify the defence related pathways and genes, regulatory elements, TFs and signaling molecules involved in imparting resistance to fungal pathogen, *Sclerotium* (Tri-partite interaction) by EMR through qRT-PCR is being done.



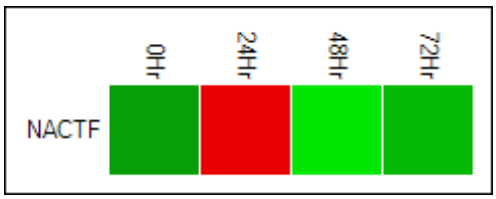
IAA production **Phosphate solublizing activity**

DNase assay

Plant growth promoting

***In planta validation* of PGP activities produced by bacterial endophytes**

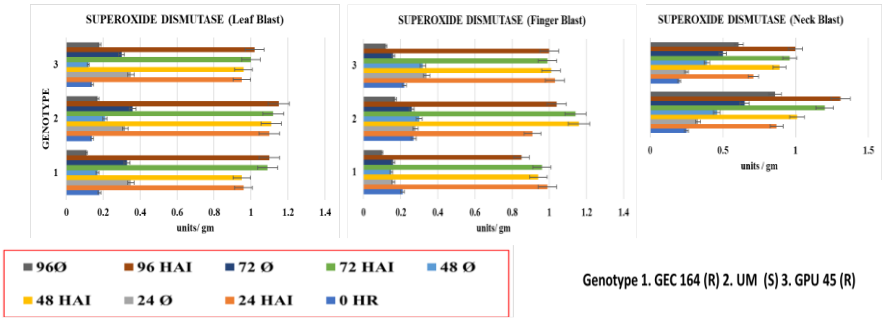
- **RT- PCR based expression analysis of selected gene under *Sclerotium* stress.**



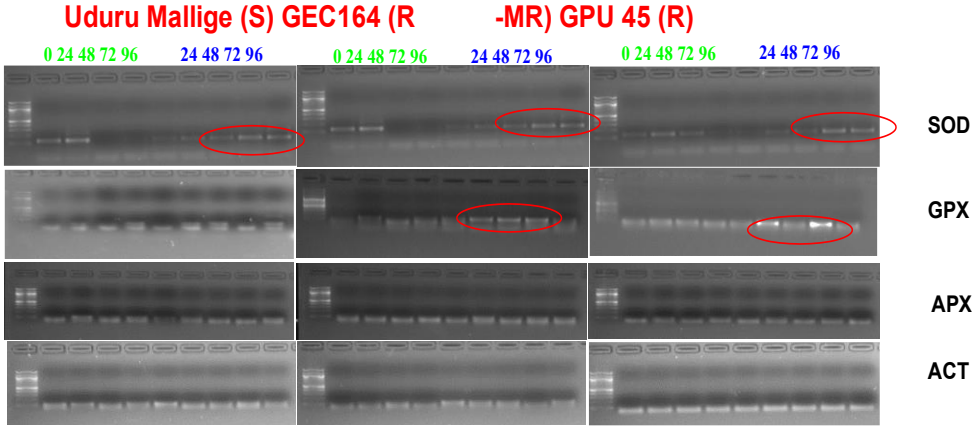
Heat map showing expression analysis of NAC TF gene

✓ **Cytological and molecular basis of organ-specific resistance to blast disease in finger millet**: Anti-oxidant enzymes activities (CAT, POX, APX, SOD) in different organs (leaf, Neck and Fingers) of finger millets after blast infection were studied. Enzyme activities significantly increased after inoculation in leaf, neck and finger infections of susceptible (Uduru mallige) and resistant (GPU45) genotypes. CAT activity was higher in susceptible line at 72 and 96 Hai in both leaf and neck but not in finger infection. APX activity was higher in leaf, neck and finger infections of resistant cultivar at 24 to 72

Hai. SOD activity was similar in leaf and neck infections of resistant and susceptible lines, however, it was significantly higher in susceptible line in finger infection

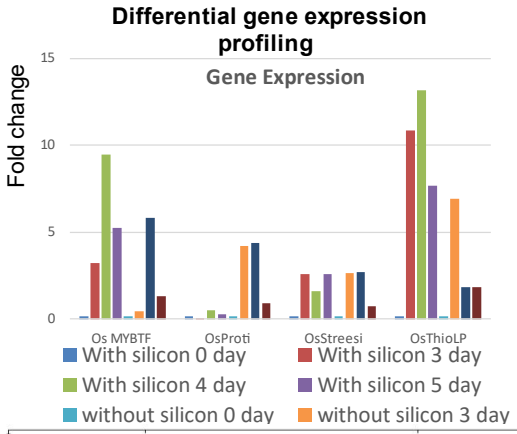


Gene Expression in finger millet genotypes against leaf blast

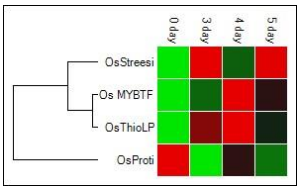


Genes for SOD and GPX were induced after *P. grisea* infection in leaves

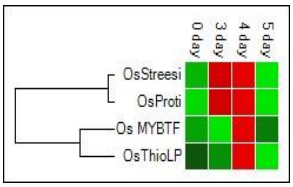
- **Deciphering silicon mediated defense against yellow stem borer in rice:** Primers were designed from the differentially expressed genes of various mechanisms, like transcription factors, stress-related kinases, thionin-like proteins, protease inhibitors, stress-related proteins, etc.RNA was isolated and cDNA was synthesized and primers were optimized for RT PCR analysis. Gene expression analysis using RT PCR showed the differential expression of genes in the silicon+, YSB+, and silicon-, YSB+ plants. Genes for MYB Transcription factor, thionin-like protein, and NMR protein was upregulated in the Silicon+, YSB+, whereas the gene such as Protease inhibitor showed downregulation.



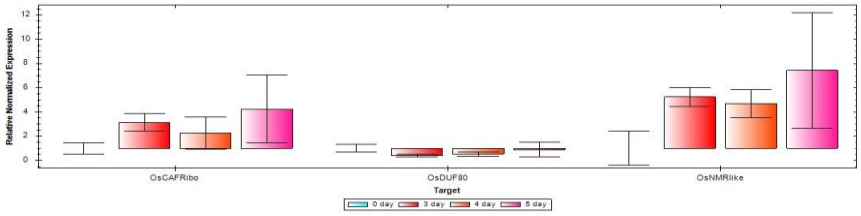
Gene expression heat map



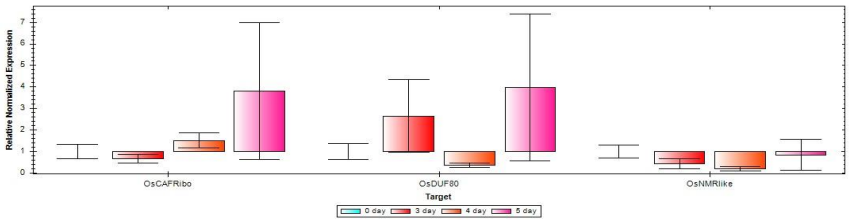
Gene expression profile in silicon + YSB infected plants



Gene expression pattern in without silicon + YSB infected plants



Gene expression pattern in silicon + YSB infected plants

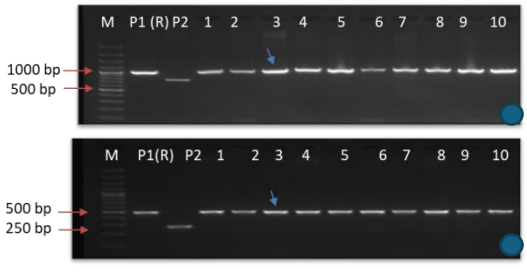


Gene expression pattern in without silicon + YSB infected plants

✓ **Development of super donors in rice carrying tolerance to multiple stresses (Bacterial Leaf Blight, Brown Plant Hopper and Blast):**

In order to develop rice lines having resistance genes for multiple biotic stress (BLB, BPH and Blast), crosses were made to introgress BLB (*Xa4, Xa5, Xa7, xa13, Xa21*) + blast (*Pi54*) and BPH (*Bph6*) genes in IRBB66 background. The presence of genes in the rice lines was confirmed using gene linked markers through MAS.. The donor lines will be useful for developing multiple stress tolerance in rice.

Crossing in rice plants and developed seeds



MAS for detection of BLB resistance genes (a) *Xa21* and (b) *xa13* using gene linked markers