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OVERVIEW

All living organisms have the ability to improve themselves through natural means in order to adapt to changing environmental conditions. However, it takes hundreds of years before any detectable improvement is obtained. Man then learned how to domesticate and breed plants in order to develop crops to his own liking and needs using various means including biotechnology. Biotechnology is defined as a set of tools that uses living organisms (or parts of organisms) to make or modify a product, improve plants, trees or animals, or develop microorganisms for specific uses. Biotechnology encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology. This monograph will focus only on agricultural crop biotechnology. The biotechnology tools that are important for agricultural biotechnology include: Tissue culture, micro-propagation, molecular breeding or marker assisted selection, genetic engineering and genome editing, molecular diagnostic tools and soil bacteriology. Agricultural Biotechnology Research Institute (ABRI) is improving important crops including cereals, fiber crops, sugarcane, fodders, oilseeds, pulses and vegetables using the above mentioned techniques. ABRI is also helping other institutes of AARI in conducting their research programs like detection of rust resistant genes in wheat through molecular markers, testing of biotech crops, incorporation of genes of various stresses in crops through genetic engineering like Roundup Ready gene, production of better somaclones, assessment of genetic diversity, screening of different genotypes for quality related genes, disease free seed multiplication etc. ABRI also has Soil Bacteriology Section which deals with microbial biotechnologies for restoration of soil and plant health. Soil microbes exert positive effects on plant growth through biological N2-fixation, P-solubilization, production of growth hormones, antibiotics and siderophore etc. Soil Bacteriology Section also functions as Bio-fertilizer Testing Lab and works on Soil-microbe-plant interactions. During 2020-21, research achievements of this institute were shared with scientific community through publication of 28 papers in international peer review journals having impact factor and recognized by Higher Education Commission (HEC), Islamabad Pakistan. Apart from these more than 100 internship students were trained in various fields of biotechnology whereas many M.Sc. and Ph.D. students carried out part of their thesis research at this institute. The detailed research objectives/achievements of this institute are discussed here.

A. GENETIC ENGINEERING

Integration of modified GT gene in *Brassica juncea* through agrobacterium mediated transformation method

Integration of modified GT gene in brassica was planned for the development of glyphosate herbicide resistance for the effective weeds control.

A total of 13150, four days old cotyledonary leaf petioles were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 162 antibiotic resistant shoots were screened on selection media containing respective antibiotic (PPT @ 3mg/L & Kanamycine @ 50 mg/L.82 putative antibiotic resistant plantlets were survived on regeneration and rooting media containing antibiotic for selection 34 putative transgenic plants were developed. All putative transgenic brassica plants were subjected to glyphosate assay and five plants found positive. Seeds were collected from those plants.

Genetic transformation of herbicide (glyphosate) resistant gene (EPSPS) in sugarcane

The objective of this experiment is the development of herbicide tolerant sugarcane plants through genetic engineering.

4200 three Weeks old calli were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 151 putative antibiotic resistant plants were screened on selection media containing Kanamycin @ 50 mg/L. 132 putative antibiotic resistant plantlets were survived on regeneration and rooting media containing antibiotic for selection. 14 putative transgenic plants were developed and their confirmation was under process. Previous year two month old putative sugarcane transgenic plants were subjected to herbicide (Glyphosate) spray trial @ 1900 ml/acre. Observations were recorded for 40 days on regular basis and four herbicide-tolerant plants were observed and their confirmation via PCR is under process.

Genetic Transformation for Herbicide Tolerant Gene in Cotton

An experiment on integration of synthetic Glyphosate Tolerance (GT) gene was planned and executed on cotton varieties viz. FH-142 and FH-490 for the development of roundup herbicide tolerant cotton plants having effective weeds control capabilities. Post pollination micro-injection of Agrobacterium culture was administrated through pollen tube pathway with intervals of 4, 8, 16 hours. A total of 1200 flowers of both genotypes were inoculated with LBA4404 agrobacterium culture having synthetic EPSPS gene using PTP transformation method. Surviving 19 putative cotton plantlets of T_1 generation were raised in seedling trays under wire house. At three leaf stage plants were sprayed with round-up herbicide with half the recommended dose. All plantlets showed susceptibility to herbicide and died. This experiment will continue into next year with optimizations in inoculation technique and herbicide dose levels. Simultaneously other in-planta gene transformation techniques will be explored next year for enhancing the chances of success in cotton transformation. The methodology of pollen tube pathway transformation is elaborated in Fig 1.

Fig 1. Process of pollen Tube Pathway transformation of Cotton.

Development of glyphosate herbicide tolerance in economically important crops (wheat) for the effective weeds control

Although weeds are a main constrain in ample crop yield, however, the un judicious use of chemical weedicides to eradicate weeds is more harmful to environment and also quite expensive for poor farmers. To thoroughly eradicate the weeds and to minimize the use of herbicides, an experiment was executed for the development of transgenic wheat harboring glyphosate resistance EPSPS under the control of constitutive promoter. Antibiotic resistant colonies of Agrobacterium AGL1 strain were confirmed through colony PCR and utilized for transformation experiment. About 1700 immature embryos of three wheat varieties viz. Akbar-19, Anaj-18 and Ujala-16 were isolated, sterilized and inoculated with synthetic EPSPS (GT) gene construct under AGL1 strain. After inoculation calli were placed on Callus inoculation media (CIM). Thirty (30) putative transgenic plants of variety Akbar-2019 were shifted to pots and their DNA was extracted. GP1 gene/promoter specific primer amplified 1250bps size fragment in 03 plants (Fig 2). Further investigation regarding the homozygosity and gene integration is needed.

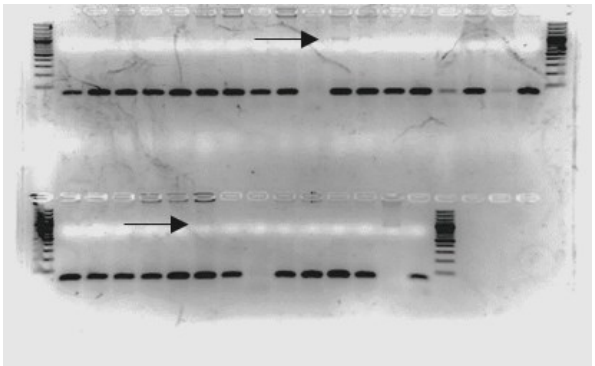

Fig 2. Herbicide tolerant transgenic events of wheat variety Akbar-19 through promoter-gene (GP-1) specific primers.

Biochemical Testing of National Coordinated Varietal Trial of Cotton and Biosafety trial

The objective of this experiment is testing of newly developed cotton varieties for insect resistance (*Cry1Ac*, *Cry2Ab*, and *Vip3A*) & herbicide tolerance (*EPSPS*) genes.

In previous year 110 entries of NCVT were received and were tested for four genes Cry1Ac, Cry2A, Vip3Aa and RR genes and quantified for Cry1Ac through ELISA. The results showed that 97 entries were positive for *Cry1Ac* and only 13 were found Non-Bt. *Cry2Aab* gene was identified in only 04 entries. RR gene was found in 25 entries and no entry was found positive for Vip3Aa gene. The samples found positive for Cry1Ac possessed Mon-531, Cry2Ab possessed Mon-15985 and RR possessed Mon-1445 (Fig 3).

Apart from NCVT 12 samples of CRS, Faisalabad were tested for 04 genes using strip test. 04 samples were collected from the farm of Minister of Agriculture and tested for 04 genes. Apart from these 06 samples were received from private sector and were tested for *Cry1Ac* gene using strip test, ELISA and PCR.

For biosafety trial is concerned we received 11 entries from different R & D organizations working on cotton as all were found positive for Cry1Ac and Mon-531 event was confirmed.

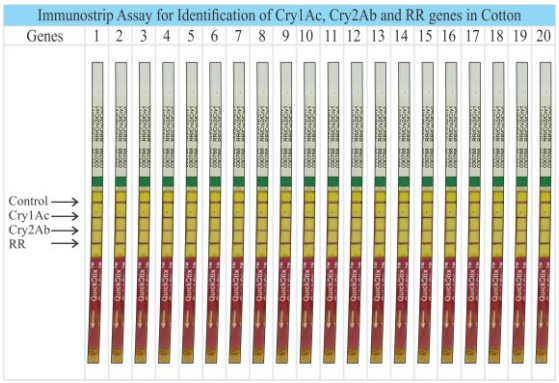

Fig 3. The results of the strip test indicating the presence of three genes i.e. *Cry1Ac*, *Cry2Ab* and *RR* genes along with positive control in each strip showing the efficacy of the strip test.

B. GENOMICS

Monitoring of GM crops by testing of GM elements through PCR techniques

The objective of the experiment is testing of two GM elements in crop plant samples for import and export purpose following International standard ISO/IEC 17025: 2017.

328 samples of different crop plants i.e. Rice, Maize, Brassica, Chickpea, Sorghum sudan grass, Peas, onion, beet, chilies, bitter gourd, bottle gourd, ridge gourd, sponge gourd, pumpkin, spinach, watermelon, tar, brinjal, sunflower, cauliflower, cabbage, okra, raddish, turnip, bajra, tomato, cucumber, squash, beans, finecut grass, cloves, sweet pepper, hot pepper etc. were analyzed for two GM elements (35S Promoter & NOS Terminator) using PCR based testing as shown in Figure 4. GMOTL has generated 3.33 Million incomes in FY 2020-21 amid COVID-19 pandemic.

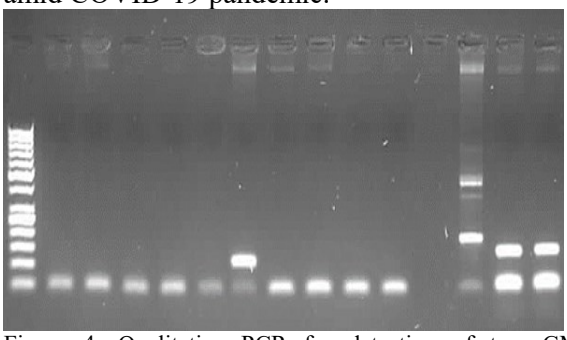

Figure 4. Qualitative PCR for detection of two GM elements i.e. NOS-118 bp terminator and CamV35S-195 bp promotor. Lane 1 is ladder 50 bp; Lane 2-7 is NOS whereas 6 is positive; Lane 8-13 are promotor whereas 13 is positive; Lane 14 and 15 are internal control of maize Phi016.

On Demand DNA fingerprinting of crops for variety registration under Plant Breeder Rights Rules

The objective of this experiment was DNA fingerprinting of various crop varieties/advance lines obtained from different government and private organizations using DNA markers through conventional PCR.

The protocol for DNA fingerprinting of crops was established as 50 polymorphic SSR markers will be used for fingerprinting using qualitative PCR and products will be visualized on polyacrylamide gel electrophoresis followed by gel documentation and data analysis. DNA Fingerprinting of 33 Genotypes of Cotton, Maize and Date Palm from different public and private organizations completed and remaining crops under progress as detailed in Table 1.

Table 1. Detail of maize and cotton genotypes whose DNA fingerprinting has been completed and reports issued to respective directorate.

Crop	Varieties / Hybrids
Maize	Hybrids: FH-988, FH-1210, FH-1914, FH-1935, YH-5395, YH-5404, YH-5427, YH-5482, YH-5561, YH-5568, YH-5569 Inbred Lines: (F-210, F-282, F-336, F-482, EL-38, EL-292, EL-115, EL-28, EL-135, EL-253)
Cotton	FH-490, FH-SUPER, MNH-1016, MNH-1020, MNH-1026, MNH-1035, Tara-CKC-333, Tara-CII-222
Date Palm	Anmol, Akhrot, Zaireen, Khudrawi
In progress	Vegetable Crops

Identification of Cytoplasmic Male Sterile and Maintainer Lines in Onion genotypes

The objective of this experiment was identification of cytoplasmic male sterile lines and maintainer lines in onion genotypes using DNA markers to facilitate Vegetable Research Institute, Faisalabad for establishment of onion hybrid system.

For these purpose two DNA markers i.e. orf725 and *ACPMS1* were used for identification of cytoplasmic and nuclear allele types in onion respectively. Both these are codominant markers which amplify CMS-S (628 bp allele), CMS-T (628 & 833 bp alleles) Fertile cytoplasm N (833 bp allele) with orf725 whereas Dominant locus (242 bp), Recessive (276 bp), Heterozygote (Both 242 and 276 bp) with *ACPMS1*. The detailed results of 21 genotypes are summarized in Table 2.

Table 2. The result of screening of 21 onion genotypes for cytoplasmic and nuclear genes for development of CMS system in onion.

Genotype	Types of Cytoplasm	Fertility restoring ACPMS1 gene
Golden ORB	Fertile	Recessive, Heterozygote
White Pearl	Fertile, CMS-T	Recessive
Red Imposta	CMS-S,	Heterozygote,
S3	Fertile	Dominant, Recessive
Phulkara	Fertile	Recessive
VRIO-2	Fertile, CMS-S	Recessive, Heterozygote
Glory	Fertile, CMS-S, CMS-T	Recessive, Heterozygote
1122 VRI	Fertile, CMS-S	Heterozygote, Dominant, Recessive
Yellow S3	Fertile	Heterozygote, Dominant, Recessive
VRIO-6	Fertile, CMS-T	Recessive, Heterozygote
T.E.G VRI	Fertile	Recessive, Heterozygote
Kessar	CMS-S, CMS-T	Heterozygote, Dominant, Recessive
Mirpurkhas S3	Fertile	Recessive
Desi Red S4	Fertile	Recessive, Heterozygote
Nasarpuri S4	Fertile	Recessive
Sultan	Fertile	Recessive
VRIO-10	Fertile	Recessive, Heterozygote
Desi Red	Fertile	Recessive
HIKE	CMS-T, Fertile	Recessive, Heterozygote
White Pearl VRI	Fertile	Heterozygote, Dominant, Recessive
Sultan Ad	CMS-T	Heterozygote
HON300A	CMS-S, CMS-T	Recessive, Heterozygote

Screening of Soybean Genotypes for Drought and heat tolerance using molecular markers.

The objective of this experiment was to assess genetic variation in 100 genotypes of soybean using DNA markers and identification of drought and heat tolerant genotypes using linked DNA markers.

The genetic diversity was assessed among 100 soybean genotypes using 33 polymorphic SSR markers. The genotypes were divided to seven distinct groups as is evident from the structure analysis give in the Fig 5.

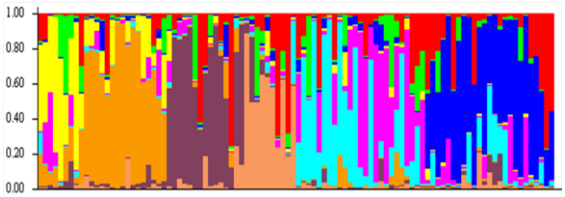

Fig 5. The structure analysis of 100 soybean accessions showing the genetic relatedness and genetic differences sketched using the binary data of 33 polymorphic SSR markers

The drought and heat tolerant genotypes of soybean were identified using linked DNA markers and most sensitive and most tolerant genotypes to heat stress are detailed below in **Table 3. Drought tolerant and drought sensitive soybean genotypes identified by use of molecular markers.**

Genotype Name	No. of Alleles	Drought tolerant/Susceptible Status
Aust942	8	Drought sensitive
FSoybean	15	Drought & Heat sensitive
Hack	15	Drought & Heat sensitive
Columbus	17	Drought & Heat sensitive
LEE	18	Drought & Heat sensitive
UFV1	18	Drought & Heat sensitive
AksarBean	18	Drought & Heat sensitive
Perry	18	Drought & Heat sensitive
KWARYGYO	23	Drought & Heat tolerant
FS60	23	Drought & Heat tolerant
Bossier	23	Drought & Heat tolerant
UDA	24	Drought & Heat tolerant
MS5	24	Drought & Heat tolerant
Pershing	24	Drought & Heat tolerant
E402	24	Drought & Heat tolerant
Willikin	25	Drought & Heat tolerant

GMO lab accreditation under ISO-17025

GMO Testing lab successfully participated once in inter lab comparison (ILC) with USDA and one time in International Proficiency Testing (PT) with FAPAS. 2nd surveillance & Re-assessment of GMO testing lab was successfully completed by PNAC officials as per guidelines of ISO/IEC-17025:2017. A total 20 NCs were raised, Corrective actions were taken and send to PNAC for evaluation. CAs were thoroughly evaluated by PNAC and found satisfactorily. The accreditation of GMO testing lab has been extended by PNAC for further three years (Fig 6). All the quality indicators like internal audit, MRMs, equipment calibrations, testing/re-testing, data recording etc. are carried out as per plan to maintain the quality of work. 72 seed/leaf samples of different crops were tested at molecular level for the presence of GM elements and reports submitted timely.


Pakistan National Accreditation Council
Ministry of Science & Technology
Government of Pakistan
Islamabad
PNAC
Certificate of Accreditation
is awarded to
GMO Testing Laboratory,
Agricultural Biotechnology Research Institute, Jhang Road,
Faisalabad-Pakistan.
in accordance with the requirements of ISO/IEC 17025:2017
The accreditation is subject to regular surveillance and compliance to the requirements of PNAC
For scope of accreditation, see Appendix
Accreditation Certificate Number: LAB 135
Date of Issue: 28-03-2021
Valid until 27-03-2024
Director General

Fig 6. Certification of scope extension of ISO accredited GMO Testing Lab of ABRI for 03 years by PNAC.

DNA markers based detection of quality related genes in spring wheat

This research work was planned to find quality related genes in spring wheat genotypes. 50 wheat genotypes of PUWYT-2020-21 were received from Wheat Research Institute. Faisalabad. Sowing were completed and leaf samples has been collected. Isolation of genomic DNA was completed and confirmed in PCR using internal control primer for wheat. 05 Linked DNA marker for wheat quality were applied on isolated DNA of all wheat entries. Marker Dx2,Dx5 was present in 46 genotypes, By8 was found in 12 genotypes, Primer Xgwm537 was present in 46 lines, Xgwm577 was found 38 lines, Xcfa2019 was amplified in 43 wheat genotypes. 07 lines showed maximum quality markers. 90 wheat leaf samples received

from WRI Fsd also screened for iron & zinc related quality markers. A representative gel image for wheat quality marker is given in Fig 7.

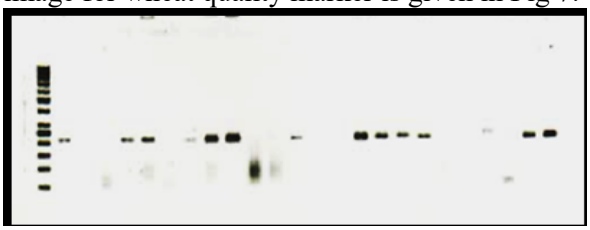

Fig 7. PCR amplification of Xgwm-537 primer linked to high molecular weight protein.

Identification of rust resistance genes in advance lines of wheat

The objective of this work was the molecular characterization of wheat genotypes to identify/tag rust resistance genes for use in the breeding program. 50 wheat genotypes of PUWYT-2020-21 were received from Wheat Research Institute. Faisalabad. Sowing were completed and leaf samples has been collected. Isolation of genomic DNA is completed and confirmed in PCR using wheat internal control primer. 08 DNA markers linked to Lr, Yr, Sr, Pm & Ltn resistance genes on 50 wheat advanced lines. 01 entries were found resistant for Lr19, 38 for Lr28, 01 resistant & 01 heterozygous for Lr34/Yr18/Pm38, 29 for Lr46/Yr29/Pm39, 25 for Lr67/Yr46/Sr55/Pm46/Ltn3 39 resistant & 03 heterozygous for Yr10. PCR for Yr5/Yr43 & Yr15 not successful Maximum no. of rust resistance genes (05) was detected in entry no 36. A representative gel image for wheat rust marker is given in Fig 8.

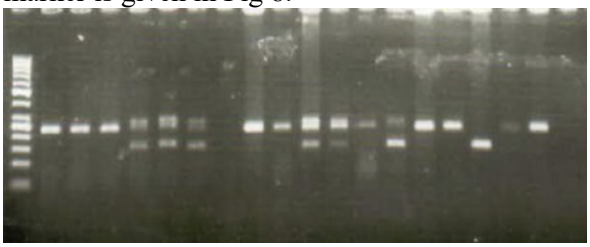

Fig 8. PCR amplification of Lr-34/Yr18/Pm38 rust primer. This gene is providing resistance collectively to leaf rust, yellow rust and powdery mildew resistance.

Rust resistance gene pyramiding in wheat using linked DNA markers

The objective of this work was the accumulation of desirable rust resistance genes from different parents to get genotypes having different combination of various Leaf, Yellow and Stem rust resistance genes. The leaf samples form previously 43 cross combination were selected from R1 (10), R-II (11), R-III (06), R-IV (04), R-V (05), R-VI (03) & A1 (04) and screened against Leaf and yellow leaf rust. 02 crosses for Lr19, 29 crosses for Lr28, 09 homozygous and 09 crosses were heterozygous for Lr34/Yr18/Pm38, 13 crosses were positive for Lr67/Yr46/Sr55/Pm46/Ltn3, 31 crosses for Lr46/Yr29/Pm39 and 13 crosses were found positive for Yr46. PCR for Yr5/Yr43 & Yr15 not successful 10 new cross combination were developed. Generation advancement for homozygous lines and development of more cross combination are continued.

Use of molecular markers for identification of Red rot disease resistance in sugarcane (*Saccharum* spp) genotypes

The yield and quality of sugarcane is affected greatly by many diseases and insects, however none more than the fungal pathogen *Colletotrichum falcatum* also known to cause redrot disease. Finding the resistance genotypes at early filial generation is one of the main objective of sugarcane breeding program. A genome based study was designed to optimize the screening of red rot disease resistant sugarcane genotypes by using molecular markers for precise detection of disease resistant material. Thirteen disease resistant genotypes viz FD-18, US-54, VMC 87/599, US-133, SP-302, US-127, CPF-246, CPF-247, CPF-248, CPF-249, CPF-251, CPF-252 and CPF-253 and 07 redrot susceptible sugarcane genotypes viz AUS-133, AUS-633, AUS-778, HSF-242, Co-1148, BF-162 and SPF-234 were obtained from Sugarcane Research Institute, AARI, Faisalabad. Twenty (20) SSR markers were applied for the differential amplification with respect to disease resistance and susceptibility. Two SSR markers mSSCIR-3 and RGA-12 uniquely differentiated redrot resistant and susceptible genotypes. A 470bps resistant band was amplified from CPF-249 and CPF-251 (Fig 9). Similarly a 980 bps resistance band was amplified from all resistant genotypes, whereas missing in susceptible genotype SPF-234. Both SSR markers can be

used for further studies for identification of redrot resistant material.

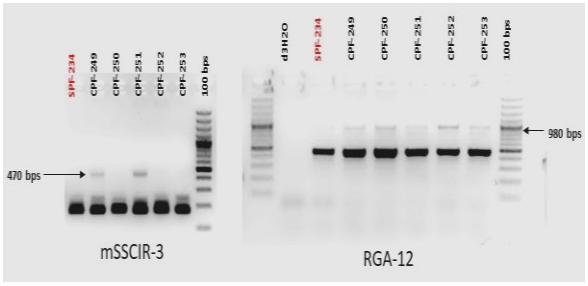

Fig 9. Amplification of unique band size with SSR marker SMC597CS identifying disease resistant sugarcane genotypes

Molecular detection of *Colletotrichum falcatum* causing red rot disease of sugarcane by using SCAR, URP and ISSR marker

The causal pathogen of redrot disease of sugarcane is *Colletotrichum falcatum*. It has many races throughout the globe. Little information is available regarding its spread mechanism and diversification under different climatic conditions and its relation to genetically dissimilar sugarcane genotypes. An experiment was planned for the identification of *Colletotrichum falcatum* races and their pattern of spread through regions and varieties on molecular basis. Diverse molecular markers like SCAR, URP and ISSR were selected from literature and synthesized. Redrot disease samples were collected from six susceptible genotypes of sugarcane and cultured on PDA media for colony development. From fungal isolates the presence of *Colletotrichum falcatum* pathogen was confirmed through ITS1 (forward) and ITS4 (reverse) primers. Marker URP13R differentiated 3 and URP17R identified 4 *C. falcatum* isolates on molecular basis.

Marker ISSR-4 and ISSR-830 identified 2 distinct *C. falcatum* strains on the basis of molecular.

Markers ISSR-830, ISSR-845 and URP17R showed that both SPF-234 and CPF-375 were infected by same *C. falcatum* strain. Marker URP17R was found to be most suitable for diversity analysis of *Colletotrichum falcatum* isolates (Fig 10).

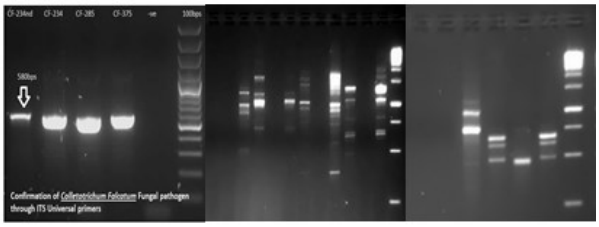

Fig 10. PCR analysis of various *Colletotrichum falcatum* isolates with molecular marker ISSR-4, ISSR-830, ISSR-845 and URP17R identifying isolates on the basis of polymorphism

C. TISSUE CULTURE

Exploitation of somaclonal variation in sugarcane for biotic and abiotic stress tolerance

The objective of the experiment is to develop drought and salt tolerant and red rot resistance somaclones of sugarcane through exploitation of somaclonal variation in combination with mutagenesis.

5680 sugarcane spindles (inner most leaf) were cultured for callus induction for development of drought tolerant sugarcane. Four weeks old (2840 calli) were treated with 0.5% EMS for 120 minutes. 236 survived regenerated calli were transferred to regeneration and multiplication media supplemented with PEG (6000). 147 plants were shifted to pots for hardening. 168 putative drought tolerant plantlets were transferred to pots for hardening (Fig 11).

For development of red rot resistance sugarcane callus of two sugarcane genotypes SPF-234 and CPF-248 were developed. The 260 plantlets were shifted to rooting medium for root development. 240 plants of SPF-234 and CPF-248 were developed and shifted in polythene bags for hardening. 300 red rot resistant plants of SPF-234 and CPF-248 were shifted in field.

Similarly for development of salt tolerant sugarcane advanced lines / varieties viz. S2005-US 658, S2006-AUS-133, CPF-249 and S2006-AUS-234 were evaluated at different salt levels to see their inherited callogenesis potential. Good callus induction was recorded in CPF-249 followed by 234 at 50 mm/L salt levels, while 133 and 658 at 100 mm/L. Callogenesis was suppressed at higher salt concentrations;

however variety/ line 249 and 234 fairly showed enough callogenesis potential even at 150 and 200 m moles / L salt level. A total of 150 somaclones were developed at different salt levels and shifted to pot for hardening.

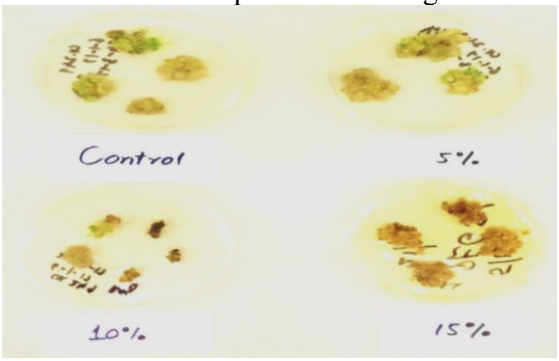

Fig 11. Selection of mutagenic calli on different PEG levels i.e. 5%, 10% and 15% along with control treatment.

Exploitation of somaclonal variation in wheat for crop improvement

Different experiments were planned for exploitation of somaclonal variation in wheat for crop improvement as discussed below.

The objective of the study was development of high yielding and stress tolerant soma clones of wheat through callus cultures derived from immature /mature embryo and development of maize mediated double haploid production in wheat. Callus was induced on MS media with different doses of 2,4D (0, 2, 4 and 6 mg/l). Data was noted for different parameters of callus. For regeneration, Kinetin and NAA were used. Twenty soma clones of wheat were transferred to pots. The seed of wheat soma clones of different crosses and varieties were sown in field as R_1 generation for testing against disease resistance and other morphological parameters.

For double haploid production wheat segregating generations (F_2 and F_3) were sown in field and used for emasculation. Four sets with 10 days intervals of two maize varieties i.e. Malka-2016 and Pearl were sown in earthen pots for maize pollens availability. Wheat spikes were taken at booting stage for emasculation and cut from base with appropriate length. One-third portion of lateral florets also cut from top to accelerate emasculation and pollination. After 3 days of emasculation, fresh maize pollens were collected in petri dish and emasculated spikes were pollinated with camel hair brush. Spikes were kept in pollinated media (40g/L Sucrose + 6% Sulfurous acid + 100mg/L 2, 4 D) for 3 days, then tillers were taken out from pollinated media and kept into growth media having 40g/L Sucrose + 6% Sulfurous acid. Developed haploid seeds placed in a petri dish then surface sterilized with 0.02% clorex for 3 minutes added two drops of tween-20, then rinsed three times with autoclaved distilled water. Haploid embryos were rescued and cultured on half strength MS media. 276 haploid embryos rescued under stereo microscope and 34 haploid plants were treated with colchicine (Fig 12).

Fig 12. Protocol optimization of maize mediated double haploid production in wheat. a) maize plants in glass house, b) pollinated spikes in incubation room, c) haploid seeds, e) haploid embryo rescued

For development of drought tolerant wheat embryos of Galaxy, Ujala and Pasban-90 were cultured for callogenesis. Maximum callus and earlier shootings success were noted in Pasban-90 followed by Ujala and Galaxy. Maximum shoots initiation were observed in MS medium having BAP (0.5mg/L) + NAA (0.25mg/L). Maximum shoots elongation were found in MS + GA3 (1mg/L) medium (Fig 13). 1/2 MS medium was found best for rooting. Total 376 somaclones were developed.

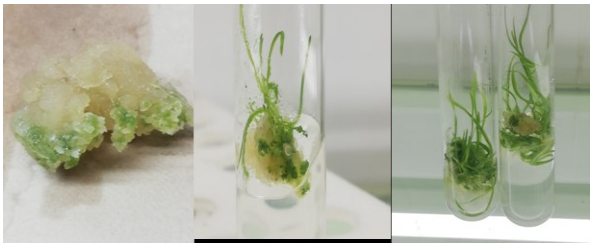

Fig. 13. Tissue culture optimization process of wheat showing callus induction, regeneration and rooting on different media in test tubes.

Optimization for micropropogation protocol in papaya

Three months old aged papaya plants were selected as explant. Apicle meristems 2cm

length was used as explant. Sterilized explants were cultured in various micro propagation media as detailed given in Table 4. Regeneration success was noted from all explant parts (apical meristem, lateral shoot bud and bud) on medium having MS + BAP (0.5mg/L) +IAA (0.25mg/L. Direct shoots with main shoot buds, shoots with apical meristem, shoots with bud, shoots detached from main bud, then shifted on various rooting media as detailed in Table 4.

Table 4. Different tissue culture media used for micro-propagation of papaya.

Sr. No.	Media Composition
1.	MS+ BAP (1mg/L) + GA3 (1mg/L)
2.	MS+ BAP (0.5mg/L) + 2iP (0.5mg/L)
3.	MS+ BAP (0.5mg/L)
4.	MS+ BAP (1mg/L)
5.	1/2MS+ BAP (0.5mg/L)
6.	MS+ BAP (1mg/L)+ IBA (0.25mg/L)
7.	MS+BAP(0.5mg/L)+ IBA (0.25mg/L)
8.	MS+ BAP(1mg/L)+ NAA (0.25mg/L)
9.	MS+ Casein Hydrolysate (300mg/L)
10.	MS+ Casein Hydrolysate (500mg/L)
11.	**MS+ BAP (0.5mg/L)+ 1AA (0.25mg/L)**
12.	MS+ BAP (1mg/L) +1AA (0.25mg/L)
13.	MS+ BAP (0.5mg/L) +1AA (0.50mg/L)
14.	MS+ BAP (1mg/L) +1AA (0.5mg/L)

Apart from above mentioned experiments different wheat generation (430 entries) were maintained and tested in various trials for evaluation of best candidate lines for sending them to provincial and national yield trials. The entries 19BT022 and 19BT002 performed best in station yield trial.

C. SOIL BACTERIOLOGY & MICROBIOLOGY

Testing of Bio-fertilizer/Bio-stimulants as reference lab for confirmation

Agricultural Biotechnology Research institute is declared as Reference Lab by the Government of the Punjab to reanalyze the 30% of the tested samples of any other challenged sample of bio-fertilizer/bio-stimulant. Our lab re-received 09 samples of bio-fertilizer/bio-stimulants till date. Results of all samples have been dispatched as summarized in Table 5.

Table 5. The statistics of the biofertilizers/bio-stimulants samples tested at the ABRI.

Brand Name	Batch number	Results
Asahi star	Asahi-010119	Fit
Restore	ADA(K-13/H/20/M	Fit
Asahi Star	ASHI-030917	Fit
Vitalus	0140118	Fit
Basfoliar Natur	4510089746	Fit
Rely	KGA/20180104-R2Y	Fit
Shakti	ITU/G/2848	Un-Fit
Restore	ADK/F/42/2018	Fit
FILIP	32EF/2017	Fit

Potential of endophytes for the growth promotion of Sunflower and Raya

Root-shoot elongation assay was conducted to check the growth promotion of crops in lab. Five isolates were selected on the basis of their efficiency. Trial was conducted in collaboration with Soil Bacteriology section AARI, Faisalabad. In a field trail, seeds of Raya were inoculated with the selected microbes and sown. The routine cultural practices were done during the growth of crop. Harvesting and threshing have been completed.

There was a significant increase in growth and yield of Raya under Endophyte inoculation compared with control. Maximum increase in yield was observed in T2 i.e. 1633. However, overall results were non-significant among treatments. A significant increase of 6.8% yield was recorded (Table 6).

Table 6. Potential of endophytes for the growth promotion of Raya crop.

Treatment	Treatment
Control	1528 ab
T1 Endophyte	1609 a
T2	1633 a
T3	1626 a
T4	1629 a
T5	1616 a

There was a significant increase in growth and yield of Sunflower under Endophyte inoculation compared with control (Table 7).

Table 7. Potential of endophytes for the growth promotion of Sunflower crop.

Treatment	Treatment
Control	1618 ab
T1 Endophyte	1719 ab
T2	1696 ab
T3	1732 ab
T4	1746 a
T5	1705 ab

Growth and yield response of field crops to PGPR and microbial synthesized metabolites

Field studies were conducted to determine the effect of PGPR and metabolites on wheat, rice and maize at ARS, Farooqabad and SSRI, Pindi Bhattian. Treatment included, control, PGPR inoculation, Kinetin @10^{-5} M, PGPR inoculation with Kinetin @10^{-5} M, Foliar Spray (FS) of PGPR metabolites, Metabolites of PGPR with Kinetin @10^{-5} M (FS), PGPR inoculation + Metabolites of PGPR (FS) (T_2+T_5) (T_7) and (T_2 + T_6) (T_8). Results of rice and wheat trials at ARS, and SSRI, revealed that PGPR inoculation + Metabolites of PGPR with Kinetin @10^{-5} M (FS) produced the highest paddy, wheat grain and maize fodder/ dry matter yield i.e., 4514, 3528 kg/ha, 4640 and 3663 kg/ha and 98.3, 21.6 t/ha, respectively(Fig 14, 15 & 16).

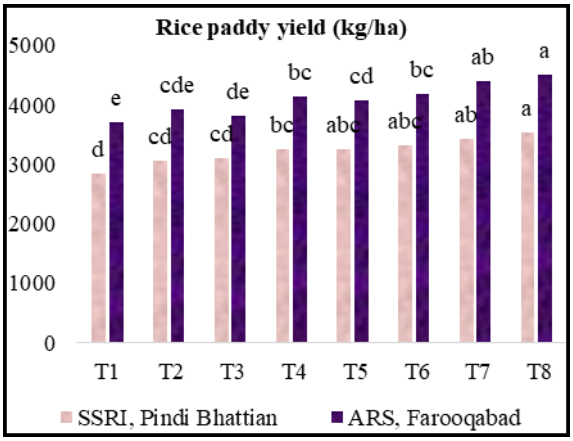

Fig. 14. Rice paddy yield at ARS and SSRI

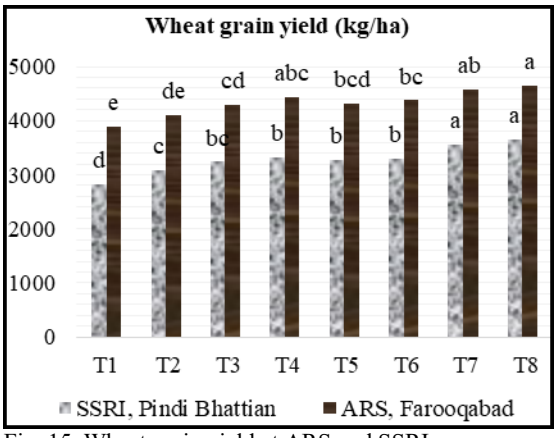

Fig. 15. Wheat grain yield at ARS and SSRI

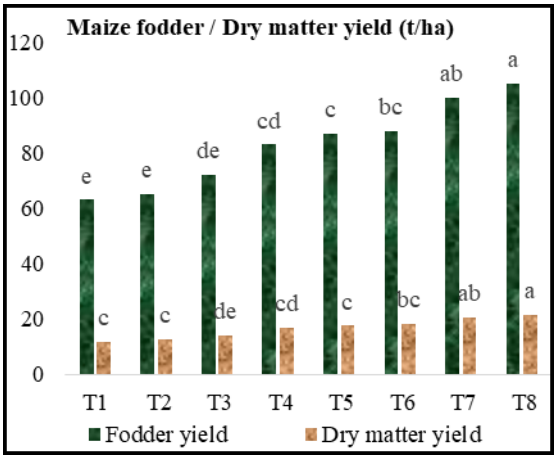

Fig. 16. Maize yield at Soil Bacteriology

Isolation, characterization & screening of biofilm producing PGPR and their effect on maize and wheat

Pot & field trials were conducted at Soil Bacteriology on maize and wheat to evaluate the effect of biofilm forming PGPR., respectively. Six treatments were employed having control and five isolates of PGPR (PGPR-1, PGPR-2, PGPR-3, PGPR-4, PGPR-5). Maize showed maximum fodder yield 518 g/pot with PGPR-3 as compared to control i.e., 310 g/pot (Fig. 17) and wheat gave maximum grain yield i.e., 4756.2 kg/ha with PGPR-4 inoculation as compared to control i.e., 3785.4 kg/ha (Fig 18)

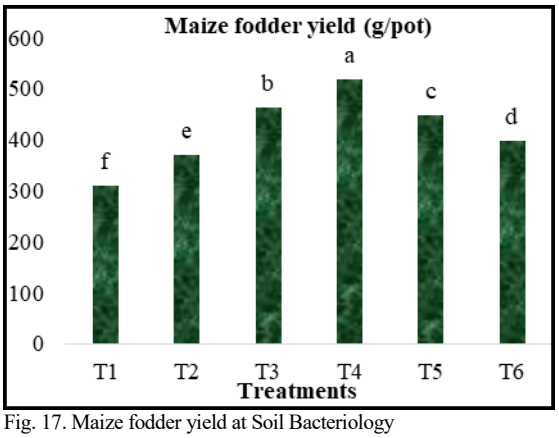

Fig. 17. Maize fodder yield at Soil Bacteriology

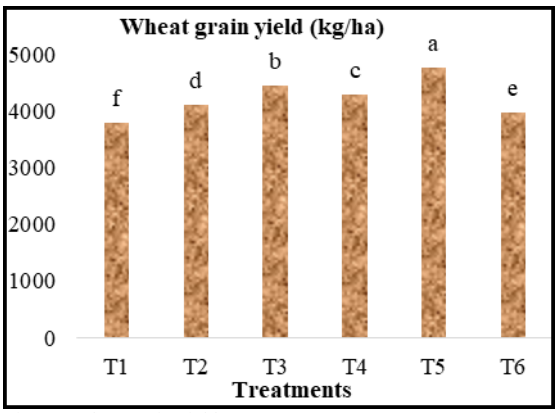

Fig. 18. Wheat grain yield at ABRI

Effect of potassium solubilizing bacterial (KSB) on enhancing growth and yield of wheat

Pot experiment at Soil Bacteriology Section was designed to evaluate the effect of K-solubilizing bacteria on growth and yield of wheat. Treatments were control, KSB-1, KSB-2, KSB-3, KSB-4 and KSB-5. Maximum grain yield was recorded in T_3 (41.6) as compared to control i.e., 28.8 g/pot (Fig. 19).

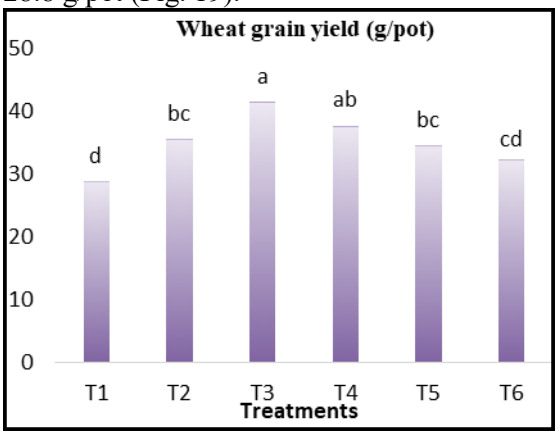

Fig. 19. Wheat grain yield at Soil Bacteriology

Mutualistic approach of *Bradyrhizobium*, PGPR & *p. Indica* on nodulation and yield of soybean

Field trial was conducted at Oilseed Research Institute to evaluate the mutualistic effect of *Bradyrhizobium*, PGPR and *Piriformaspora indica* inoculation on growth and yield of soybean. Treatments were control, *Bradyrhizobium* sp), PGPR inoculation *Piriformaspora indica* inoculation, T_2+T_3 (T_5), T_3+T_4 (T_6), T_2+T_4 (T_7), T_2+T_3+T_4 (T_8). Results showed that maximum grain yield (2933 kg/ha) was obtained from T_8 treatment as compared to control (2196 kg/ha) (Fig. 20).

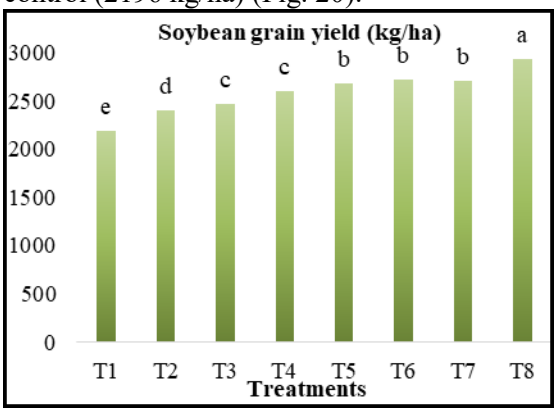

Fig. 20. Soybean at Oilseed Research Instt.

Isolation and characterization of siderophore producing bacteria and their effect on crop production

Field experiment was conducted at ISCES and ABRI to check the efficiency of siderophore producing bacteria in improving growth and yield of maize and wheat crop, respectively. Treatments were control, SPP-1, SPP-2, SPP-3, SPP-4, SPP-5, SPS-9 and SPS-10. Whereas, SPP-3 and SPP-5 gave highest maize grain yield (6026.7 and 6813.1 kg/ha) as compared to control (4173.0 kg/ha) Fig.21. Results indicated that SPP-3 and SPP-5 gave highest wheat grain yield (4600 and 4580) as compared to control (3326.7 kg/ha) (Fig. 22).

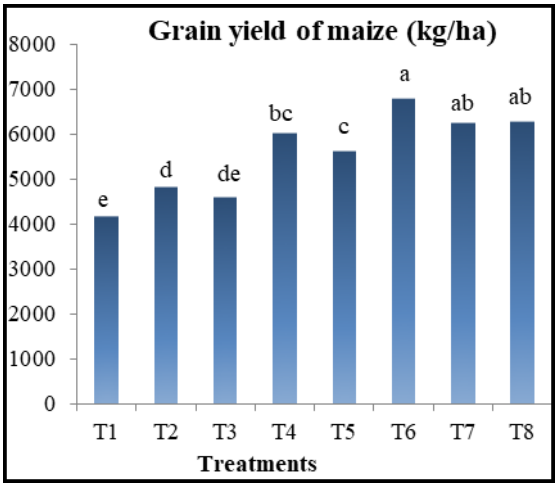

Fig. 21. Grain yield of maize at ISCES

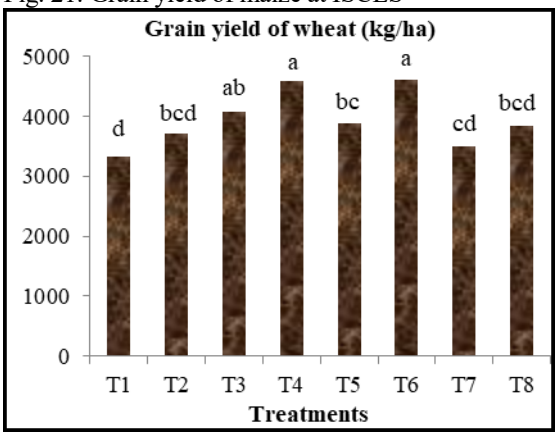

Fig. 22. Grain yield of wheat at ABRI

Interactive effect of physiological precursor and microbial inoculum on the yield of legumes

Field studies were conducted at PRI and ABRI, Faisalabad on mungbean and chickpea, respectively to assess the effect of Rhizobium enriched culture withL-tryptophan (L-TRP) & L-Adenine (L-ADE) to improve yield of legumes. Treatments were control, *Rhizobium sp.*, L-TRP @10^{-5} M, L-Adenine @ 10^{-5} M, *Rhizobium* + L-TRP @ 10^{-5} M, *Rhizobium*+L-ADE @ 10^{-5} M. Results revealed that *Rhizobium* + L-ADE @ 10^{-5} M gave highest mung bean grain yield (1279.7 kg/ha) as compared to control (1107.9 kg/ha) (Fig. 23). Results exposed Rhizobium in combination with + L-Adenine @ 10^{-5} M gave highest grain yield (1520.0 kg/ha) as compared to control (1033.3 kg/ha) (Fig. 24).

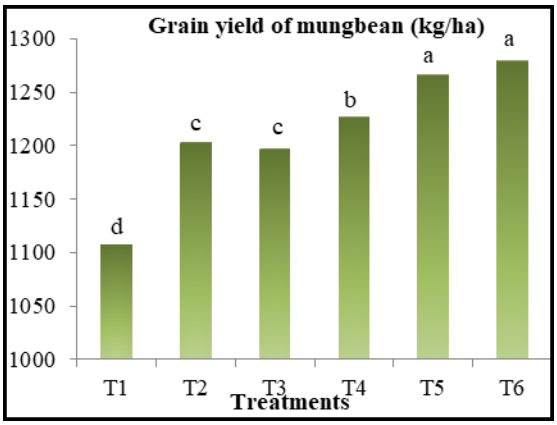

Fig. 23. Grain yield of mungbean at PRI

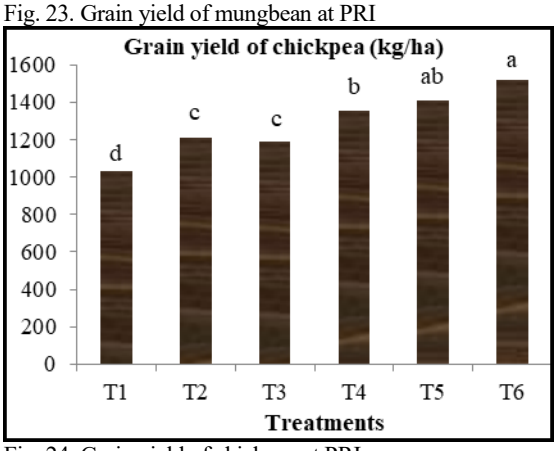

Fig. 24. Grain yield of chickpea at PRI

Growth and yield response of oil seed crops to PGPR and microbial synthesized metabolites

Field trial was conducted on Raya at ABRI to study the role of Secondary Plant Metabolites (SPMs) in plants' survival and establish ecological relationships. Treatments were control, PGPR-Azotobacter sp., PGPR-Pseudomonas sp., Metabolite spray, Metabolite spray + PGPR -Azotobacter sp., Metabolite spray + PGPR-Pseudomonas sp. Result showed that bacterial inoculation along with metabolite spray showed significant increase in yield. The treatment T_6 produced maximum yield i.e., 2266 kg/ha in Raya and significantly higher than control (Fig. 25).

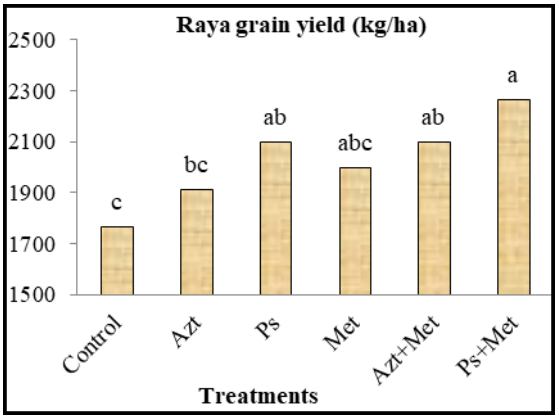

Fig. 25. Grain yield of Raya at ABRI

Screening of crop specific exopolysaccharide producing PGPR from drought prone areas of the Punjab

Field study was conducted at the Soil Bacteriology Section and ABRI, on maize and wheat, respectively to study the role of exopolysaccharides (EPS) producing bacteria. Treatments were control, CH-2 B, CH-3A, CH-4A, PS-2A, PS-2B and PS-2D. Result showed that inoculation with bacterial isolates (CH-2B) showed significant increase in yield in both maize and wheat. The treatment T_2 produced maximum maize fodder yield i.e., 85 t ha^{-1} and in wheat grain yield i.e., 4247 kg/ha (Fig. 26-27).

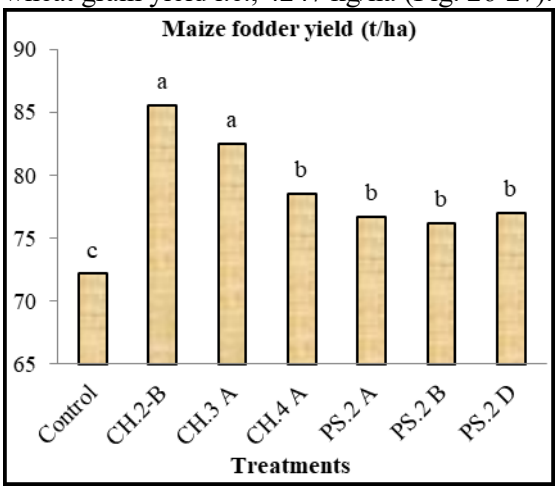

Fig. 26. Maize fodder at Soil Bacteriology

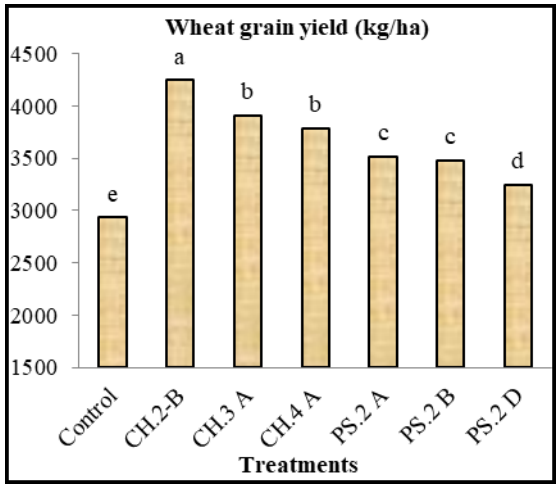

Fig. 26. Wheat grain yield at ABRI

Co-inoculation of *Bradyrhizobium* and phosphate solubilizing microbes on growth promotion of groundnut under rain-fed conditions

A field trial was planned to evaluate the efficiency of *Bradyrhizobium* and P solubilizing microbes (PSM) on groundnut under rain-fed conditions at SAWCRI. Treatments comprised of control and three isolates of *Bradyrhizobium* along with PSM and their combination. Result showed that *Bradyrhizobium* isolate-2 and PSM (T_7) gave highest (1020 kg/ha) grain yield (Fig. 27).

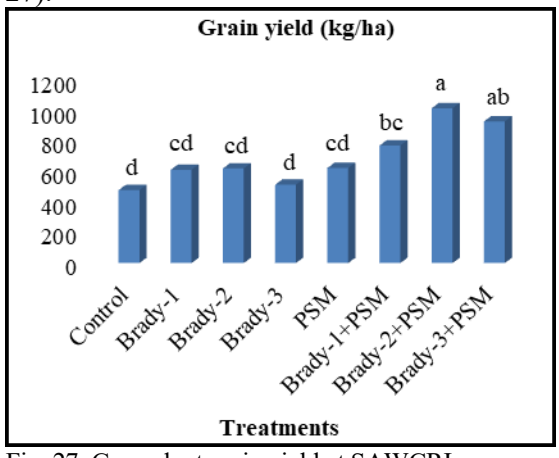

Fig. 27. Groundnut grain yield at SAWCRI

Plant microbe interactions for the growth ad yield improvement of Lucerne fodder

In a field study on Lucerne at Fodder Sub-station, treatments were control, Rhizobium, PGPR inoculation, co-inoculation, Tryptamine (TRY) @ 10^{-5} M Foliar Spray(FS), Rhizobium + TRY @ 10^{-5} M (FS), PGPR + TRY @ 10^{-5} M

(FS), Co-inoculation + TRY @ 10^{-5} M (FS). Result showed that co-inoculation (Rhizobium + PGPR) with TRY(FS) gave highest fodder yield 112.9 t/ha significantly higher than control (Fig. 28).

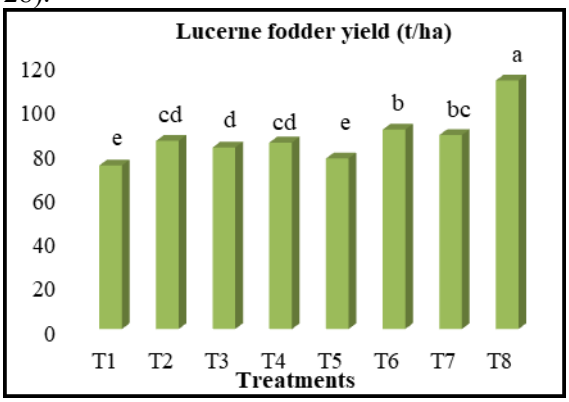

Fig. 28. Lucerne at Fodder Sub-station

Effect of different auxin precursors with pgpr inoculation for the growth promotion of wheat and maize

Field studies were conducted at the Soil Bacteriology Section and ABRI. Treatments were control, PGPR Inoculation, Tryptamine (TRY) @ 10^{-5}M, L-Tryptophan (L-TRP) @ 10^{-5} M, PGPR inoculation + TRY @ 10^{-5} M, PGPR + L-TRP @ 10^{-5} M. PGPR + TRY gave maximum maize fodder and wheat i.e., 73.7 t/ha and 4420 kg/ha, respectively (Fig. 29-30).

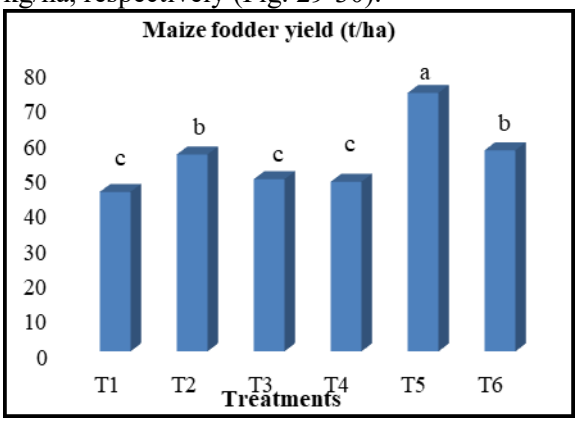

Fig. 29. Maize fodder yield at Soil Bacteriology

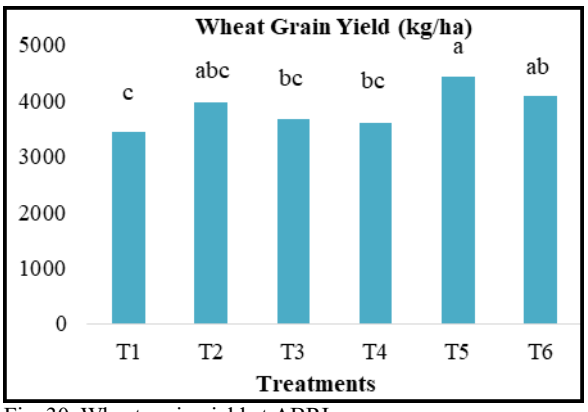

Fig. 30. Wheat grain yield at ABRI

Comparative study of various methods of application of biofertilizer for growth improvement of cereals

In a pot trial best biofertilizers' application method was tested at Soil Bacteriology. Eight treatments viz; Control (no inoculum),Seed coating, Flooding, Foliar Spray, T_5: T_2+T_3, T_6: T_2+T_4, T_7: T_3+T_4 & T_8: T_2+T_3+T_4 were used. Treatment 'seed application' proved better in both cereals followed by 'Seed application+ flooding + foliar spray'. In general, inoculum application proved better than no inoculum (control) (Fig. 31, 32).

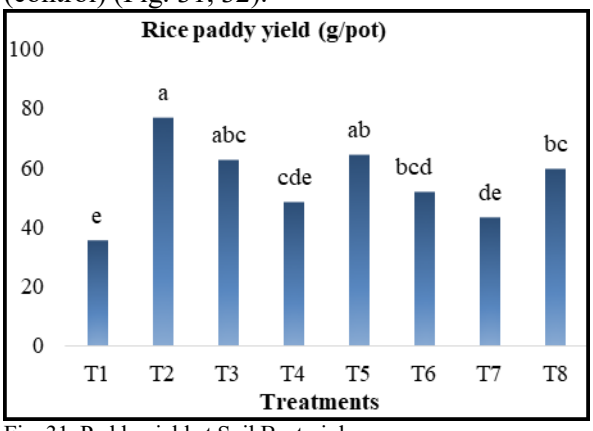

Fig. 31. Paddy yield at Soil Bacteriology

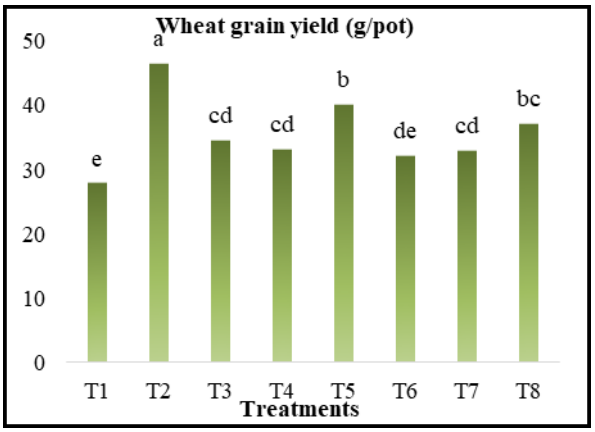

Fig. 32. What grain yield at Soil Bacteriology

Effect of various crop residue management strategies on soil health in rice-wheat cropping system

Study was conducted to note the ill effects of crop residue burning on soil health and environment. The burnt and unburnt soil samples were collected by Soil Fertility field staff from various districts of the Punjab and 240 + 36 samples were processed and analyzed at Soil Bacteriology Lab for microbial count using dilution plate technique and soil organic carbon as per standard procedure. Results of burnt vs. unburnt soil samples after rice and wheat harvest showed significant reduction in microbial population expressed as CFU per gram of soil. Measurable reduction in total organic carbon (TOC) because of crop residue burning was observed. Reduction in average microbial count by 15% and TOC by 10-15% was observed in the burnt samples. (Fig. 33, 34).

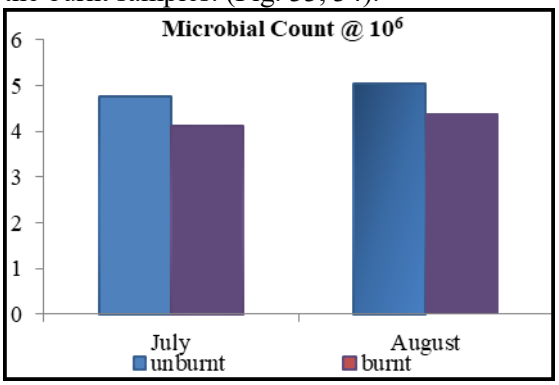

Fig. 33. Variations in microbial count due to burning of crop residues during 2020.

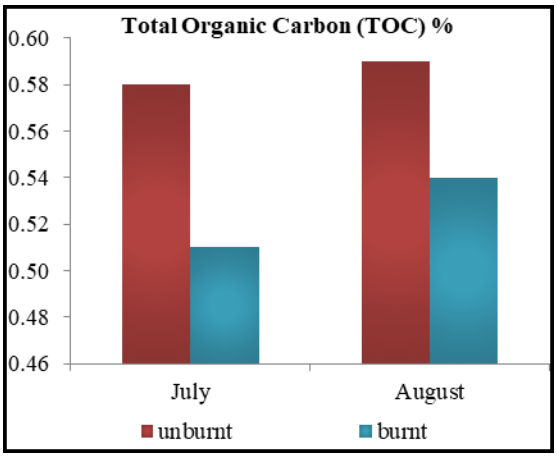

Fig. 34. Variations in total organic carbon due to burning of crop residues during 2020.

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- **Miscellaneous:**

1.	Radio Talks Delivered	02
2.	Visits of Delegations	30
3.	Training Seminars Delivered	25
4.	Class visits	20
5.	Internship Students	100
6.	Sample analyzed	328
7.	Income Generation	3.4 M

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