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Annual Report (Year 2022-23)

OVERVIEW

Biotechnology is a multidisciplinary field of science and technology that involves the use of living organisms, cells, and biological systems, or their derivatives, to develop or create products, improve processes, or solve problems. It encompasses a range of techniques and methodologies from various scientific disciplines, including molecular biology, genetics, microbiology, biochemistry, and bioinformatics. The Agricultural Biotechnology Research Institute (ABRI) employs essential tools such as tissue culture, micro-propagation, molecular breeding, genetic engineering, genome editing, molecular diagnostics services for crop improvement. ABRI is centered on enhancing cereals, fiber crops, sugarcane, fodders, oilseeds, pulses, and vegetables yields, contributing to research programs in other institutes like rust-resistant gene detection in wheat, biotech crop testing, incorporation of stress-resistant genes through genetic engineering (e.g., Roundup Ready gene), somaclone production, genetic diversity assessment, quality-related gene screening, disease-free seed multiplication, and microbial biotechnologies for soil and plant health restoration. The Soil Bacteriology Section at ABRI Soil Bacteriology Section deals with soil microbes exert positive effects on plant growth through biological N_2-fixation, P-solubilization, production of growth hormones, antibiotics and siderophore etc. Soil Bacteriology Section also functions as designated Biofertilizers Testing Lab. The ABRI hosts two ISO 17025:2017 accredited labs i.e. GMO testing Lab and Biofertilizers Testing Lab. During the year, 2022-2023, **15,952** microbial inoculants were provided to the farmers for improving their soil health. In the academic realm, ABRI disseminates its research through the publication of 28 papers in international peer-reviewed journals recognized by the Higher Education Commission (HEC), during 2022-23. Moreover, the institute plays a crucial role in education and training, having mentored over 100 internship students in various biotechnological fields and hosted M.Sc. and Ph.D. students conducting thesis research. These comprehensive research objectives and achievements highlights ABRI's pivotal role in advancing agricultural biotechnology and improvement of crops and soil health.

A. DIAGNOSTIC

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 95 entries of NCVT were received and 145 samples were tested for four genes *Cry1Ac*, *Cry2A*, *Vip3Aa* and *RR* genes (Fig. 1). The results showed that 106 samples were positive for Cry1Ac, 27 for Cry2Ab and 44 for RR gene whereas 10 found non-BT entries were positive for *Cry1Ac* and only 13 were found Non-Bt. Further, 85 samples were single gene, 15 were double gene, 35 were triple gene and 10 were Non-Bt. (Table 1).

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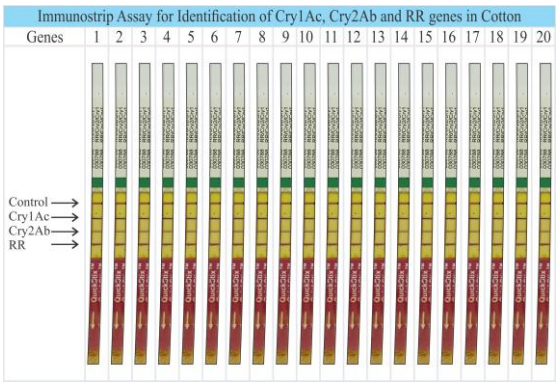

Fig 1. The results of the strip test indicating the presence of three genes i.e. *Cry1Ac*, *Cry2Ab* and *RR* genes along with positive control in each strip showing the efficacy of the strip test.

Table 1. National Coordinated Cotton Testing Results: Detection of Cry1Ac, Cry2Ab, Vip3A, and RR genes using strip test and PCR Analysis.

Gene Name	Positive Sample	Event
Cry1Ac	106	MON-531 only
Cry2Ab	27	MON-15985 only
RR	44	MON-1445 only
Vip3A	0	-
Non-Bt	10	-

Apart from that different samples were received from private sector (Saim Seeds, CABI, Sanifa, NIAB and Atta Seeds) (Table 2) and public sector (Table 3) for identification of *Cry1Ac*, *Cry2Ab*, *Vip3A*, and *RR* genes.

Table 2. On demand testing of cotton samples received from various private sectors for *Cry1Ac*, *Cry2Ab*, *Vip3A*, and *RR* genes using strip test and PCR Analysis.

Source	Sample Name	Results
Saim seed services	Saim-32, Saim-102	Positive for Cry1Ac only
CABI	1-16	01 triple gene, 07 *Cry1Ac* only & 08 Non-Bt
Sanifa Agri. Services	SAS-1, SAS-2	Both Positive for Cry1Ac only
NIAB	NIAB-993, NIAB-2008	Non-BT
ATTA seeds	AS-85, Captian-200 Captain-300	AS-85 (*Cry1Ac*) Captian-200 & Captain-300 (triple genes)

Table 3. On demand testing of cotton samples received from various public sector organizations for *Cry1Ac*, *Cry2Ab*, *Vip3A*, and *RR* genes using strip test and PCR Analysis.

Source	Sample Name	Result
CRS, Faisalabad	1-295	163 triple gene 112 double gene & 20 Single gene
NIAB, Faisalabad	1-300	134 triple gene 96 double gene, 34 single gene and 36 Non-Bt.

B. GENOMICS

Genome-wide association studies (GWAS) to reveal the genetic architecture of grain yield under heat stress condition in maize

The objective of this experiment was development of sequencing data base of 300 maize genotypes for variety protection under Plant Breeders Rights Rules and Candidate's gene mapping to identify HS tolerance genes in local germplasm for development of functional markers for HS responsive genes in maize.

Different maize genotypes (300) collected from AJK, KPK, Punjab and NARC Islamabad and germplasm collection of Maize and Millets Research Institute, Sahiwal were sown under normal and heat stress conditions at Faisalabad and Sahiwal and evaluated for different morphological, physiological and biochemical traits followed by genotyping by sequencing from Shanghai Biozeron company. Data analysis is carried out of morphological traits. The correlation analysis depicted strong association of grain yield with cob length, grain length, number of grains and hundred grain weight (Fig 2).

CRISPR/CAS9-mediated knock out of FON4 gene in rice for induction of multi-floret character

The objective of the experiment is to knockout FON4 gene in rice for development of Multi-floret character to harvest more number of grains per floret. Our target was to silence the FON4 gene in rice for production of multifloret rice genotypes. The vector and gRNA was designed with the vector for FON4 gene

(Gene ID 107276890) using Benchling suite (https://benchling.com) (Fig 3).

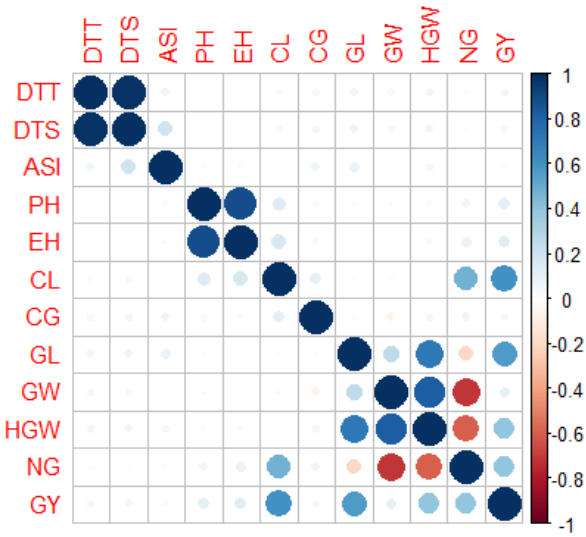

Fig 2. Correlation analysis of different morpholgical traits of 300 maize genotypes planted under Heat Stress at Maize and Milets Research Institute, Faisalabad. DTT; Days to tasseling, DTS; Days to silking, ASI; Anthesis-Silking Interval, PH; Plant height, EH; Ear height, CL; Cob length, CG; Cob girth, GW; Grain width, HGW; Hundred grain weight, NG; number of grains and GY; grain yield.

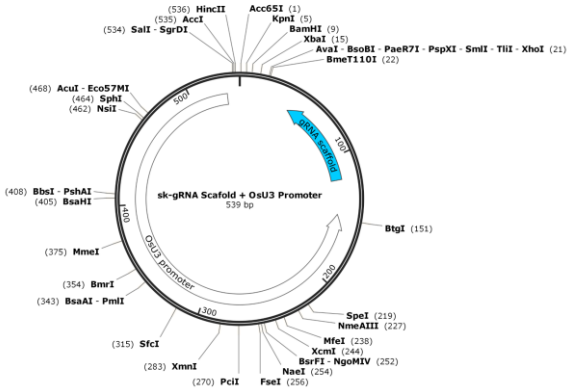

Fig 3. Selection of target site, designing of gRNA and synthesis of vector for knocking out of *FON4* gene in rice.

On demand DNA fingerprinting of wheat, maize and vegetable crops for variety registration under plant breeders rights

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 4.

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. 60 wheat genotypes of PUWYT-2022-23 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. 09 Linked DNA marker for wheat quality were applied on isolated DNA of all wheat entries. Marker Dx2/Dx5 was present in 47 genotypes, By8 was found in 29 genotypes, Xgwm537 was present in 52 lines, Xgwm577 was found 51 lines, Xcfa2019 was amplified in 35 wheat genotypes, Xgwm291 was found 60 lines, Xbarc144 was found 45 lines, WMC331 was found 60 lines and Xgwm359 as present in 19 entries. 06 lines showed maximum quality markers (Fig 4).

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. 60 wheat genotypes of PUWYT-2022-23 were received from Wheat Research Institute. Faisalabad.

Total cellular DNA was isolated from leaf samples and exposed to PCR for the identification of rust resistant gene. 10 DNA markers linked with Leaf rust, Yellow rust, Stem

rust, powdery mildew and leaf tip necrosis were applied on isolated DNA in PCR.

Table 4. List of crop varieties DNA fingerprinted during FY 2022-23.

Crop	NO.	Genotypes
Citrus	11	Kumquart, Senguinella, Ruby Red, Blood Red, Valencia late, Succari, Musambi, Kaghazi Lime, Sweet lime, Eustis Lime, AARI Khatti
Rice	10	KSK111H, Basmati-385, IR58025A, PK10683, Al-Khalid, SRI25, SRI28, Super Basmati, Super Basmati 19, Basmati-370
Maize	07	Hybrids: MNH-06, MNH-20, MNH-56, FH-1884, FH-1954, YH-5560, 2025 (Variety)
Barley	07	Jau-17, Sultan-17, Jau-21, Peral-21. Talbina-21 & B-18009, E-21
Tomato	04	Samar-F1, Marjan-F1, LTTH-904, LTH-324
Pea	04	Peas-09, Metor, Supreme, 18B003
Sugarcane	04	YTFG-236, HSF-240, SPF-234, CP-77-400
Ber	04	Akash, Anokhi, Moon, Umran
Mung bean	04	MPP-15024, AZRI Mung 2020, Bahawalpur Mung 2017 and AZRI Mung 2018
Groundnut	04	10AK002, BARI-2016, BARI-2011 & Fakhr-e-Chakwal
Wheat	05	HYT-70-16, 17086, 18012, D-18721, D-17728
Potato	03	Kashmir (FD73-44), Sutluj (FD76-59), FD-7438
Onion	03	Phulkara, Sultan, Pearl White, VRI0-14
Brassica	03	RBN-18021, RBN-18006, RBJ-15013
Sesame	03	Anmol Till, AARI Till, ORI White
Soybean	03	NBG-SOY-N312, ORI-SOY-25, ORI-SOY-91
Mash bean	03	Chakwal Mash and Barani Mash, 14CM-7005,
Safflower	03	Thori-78, Saff-65, SAF-111
Sunflower	02	FH-741, FH-751
Turnip	02	Purple Top, Purple Top Agata
Tar	02	Punjab-Tar-1, Tar-local
Okra	02	Okra-1900, P-21
Total	93	

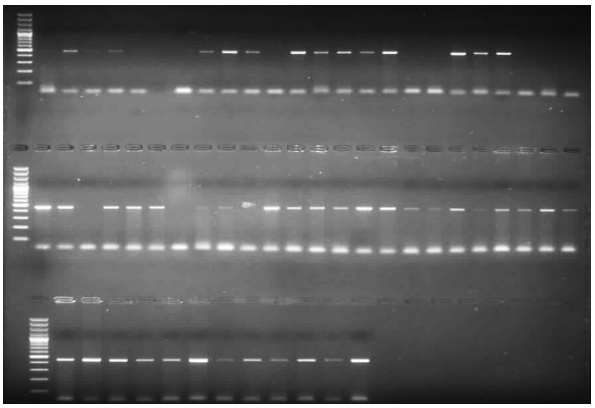

Fig. 4: PCR amplification of Dx2/Dx5, linked marker for wheat gluten

It was found that 01 lines was positive for Lr19, 42 for Lr28, 24 for Lr29, 02 resistant & 02 heterozygous for Lr34/Yr18/Pm38, 19 for Lr46/Yr29/Pm39, 28 for Lr67, 17 for Lr68, 38 for Yr5, 15 for Yr10 while PCR for Yr15 was not successful. Maximum no. of rust resistance genes i.e. 06 were found in entries no 13, 33 & 36 (Fig 5).

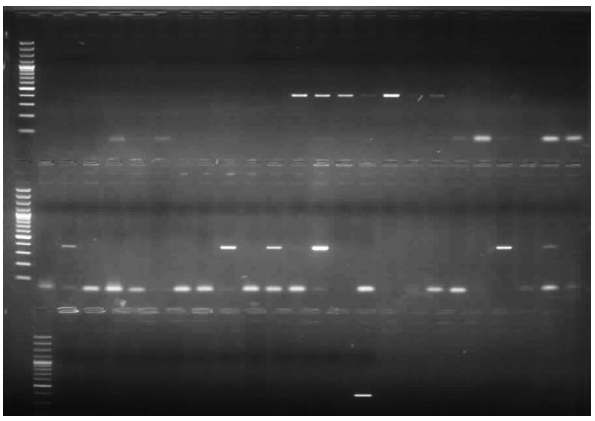

Fig. 5: PCR amplification of csGS, leaf rust resistant marker linked with Lr68 gene.

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

Disease-causing organisms can significantly decrease the productivity of sugarcane plants and sugar quality. Among the disease-causing organisms, *Colletotrichum falcatum* Went causes the most significant economic loss (5–50%) in the sugarcane production

due to red rot disease. This loss results in only 31% sugar recovery. It is reported that *C. falcatum* can kill sugarcane plants. Very little information is available regarding its spread mechanism and diversity under various climatic zones and 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 international literature and synthesized. DNA was isolated from fungal colonies taken from CP-234, US-285, US-375, AUS-832. To confirm presence of *Colletotrichum falcatum* ITS primers ITS1 as forward and ITS4 as reverse were deployed. Band of 580bps confirms infection of *C. falcatum* pathogen. Marker ISSR-845 and ISSR-830 demonstrated that CP-234 and US-375 were infected by same *C. falcatum* strain. *C. falcatum* strains from same location tend to infect different host sugarcane genotypes. There may also be resistant genes present in genotypes that make them resistant to one type of *C. falcatum* strain (Fig-1).

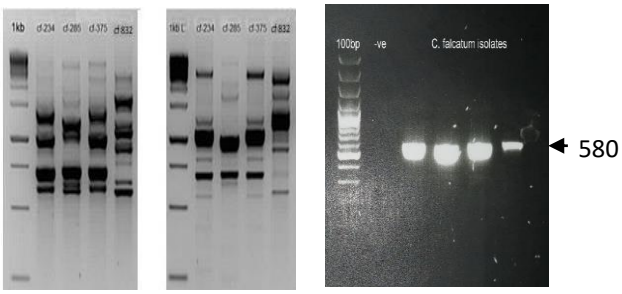

Fig 6. PCR analysis of various *Colletotrichum falcatum* isolates with ITS primers and molecular marker like ISSR-830, ISSR.-845 to identify fungal isolates on the basis of polymorphism.

In-vitro studies of morphometrics of *Colletotrichum falcatum*, a red rot disease causing pathogen in sugarcane

The experiment was designed to study the ecological variations in morphology of sugarcane red rot pathogen, Colletotrichum falcatum, isolated from infected sugarcane samples collected from different areas/districts (Bahawalpur, Bahawalnagar and Rahim Yar Khan, Chiniot, Faisalabad, Toba Tek Singh, Jhang, Khushab, Sargodha, Bhakkar) of the Punjab. Red rot infected sugarcane samples (30 samples from each) were collected. Isolation and purification of *C. falcatum* was carried out on Potato Dextrose Media (PDA). Based on studied morphometrics of the pathogen i.e. Mycelium (growth diameter), Colony characters, color (light grey, dark grey), texture (fluffy, rough), culture appearance (thin, thick), Sporulation (very fast, fast, slow), Shape of conidia (sickle shaped with tapering edge/round edge, fusoid shaped with tapering edge/round edge), 3 isolates/strains were found to be present and infecting sugarcane crop of observed districts in the Punjab and detected isolates/strains were recorded from the different and as well as from the same single sugarcane variety Fig 7.

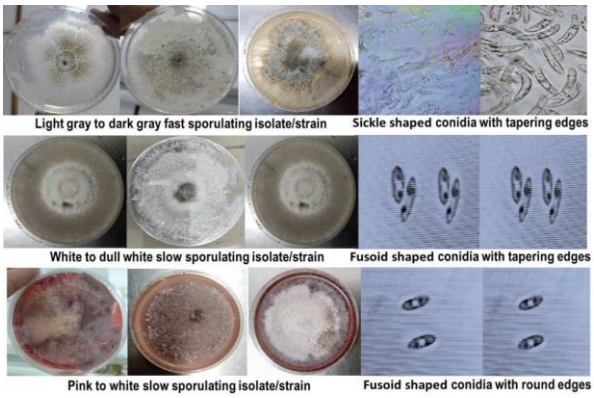

Fig 7. Cultural variation in morphology of *Colletotrichum falcatum*, a red rot disease causing pathogen of sugarcane crop of the Punjab.

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.

A total of 06 seed samples (including PT & ILC) of different crops were analyzed for two GM

elements (35S Promoter & NOS Terminator) using PCR based testing as shown in Figure 8.

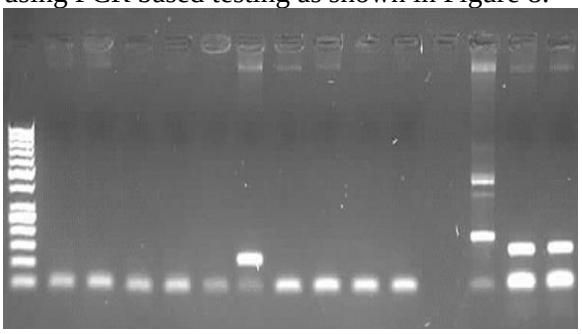

Figure 8. Qualitative PCR for detection of two GM elements i.e. NOS-118 bp terminator and CamV35S-195 bp promotor. Lane 1 is ladder 50 bp; Lane 2-7 is NOS whereas 6 is positive; Lane 8-13 are promotor whereas 13 is positive; Lane 14 and 15 are internal control of maize Phi016.

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

Red rot disease is characterized by interrupted red and white patches within the cane along with a pungent alcoholic odour when the cane is split open. Caused by the fungus *Colletotrichum falcatum*, a soil born pathogen that severely reduces the quality and yield of sugarcane. Finding the resistance genotypes at early filial generation is one of the main objectives of sugarcane breeding program. A genome based experiment was designed to optimize the screening of red rot disease resistant sugarcane genotypes by using SSR based molecular markers for efficient and accurate screening of disease resistant and susceptible sugarcane genotypes. Four disease resistant cultivars viz. CP-77-400, CPF-251, CPF-252, CPF-253 and two red rot susceptible genotypes viz. HSF-240 and SPF-234 were obtained from Sugarcane Research Institute, AARI, Faisalabad. Ten (10) new SSR markers were applied for the differential amplification with respect to disease resistance and susceptibility. Two SSR markers SMC7CUQ and SMC36BUQ uniquely differentiated redrot resistant and susceptible genotypes on the basis of amplified fragment sizes. Marker SMC7CUQ gave a single unique band of 210bps in redrot susceptible variety SPF-234. This band was absent in redrot resistant genotype. Marker SMC36BUQ amplified two DNA fragments in susceptible genotype HSF-240 (125 and 130bps) whereas same marker amplified 2 distinctive bands in resistant genotype CP-77-400 (135 and 150 bps). Both SSR markers are good candidate for further studies on identification of redrot resistant genotypes (Fig 9).

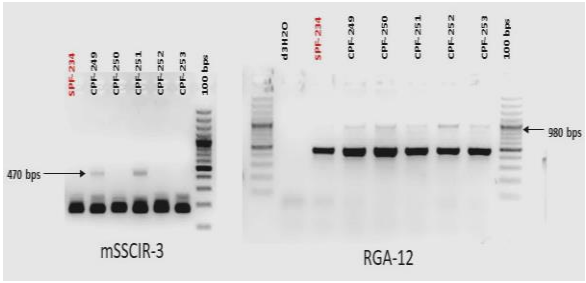

Fig 9. Amplification of unique band size with SSR marker SMC597CS identifying disease resistant sugarcane genotypes

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.

4210 sugarcane spindles (inner most leaf) were cultured for callus induction. Four weeks old (3560 calli) were treated with 0.5% EMS for 120 minutes (Fig 10). 310 survived regenerated calli were transferred to regeneration and multiplication media supplemented with PEG (6000) (Fig 11). 270 plants were shifted on rooting media. 59 plants were shifted to pots for hardening. 26 putative drought tolerant plantlets were shifted to field for field evaluation.

For development of red rot resistance sugarcane callus, the 1000 plantlets were shifted to rooting medium in test tubes/jars for root development. 700 plants of CPF-248, CPF-251 and CPF-252 were developed and shifted in polythene bags for hardening. 500 plants of CPF-248, CPF-251 and CPF-252 were shifted in field.

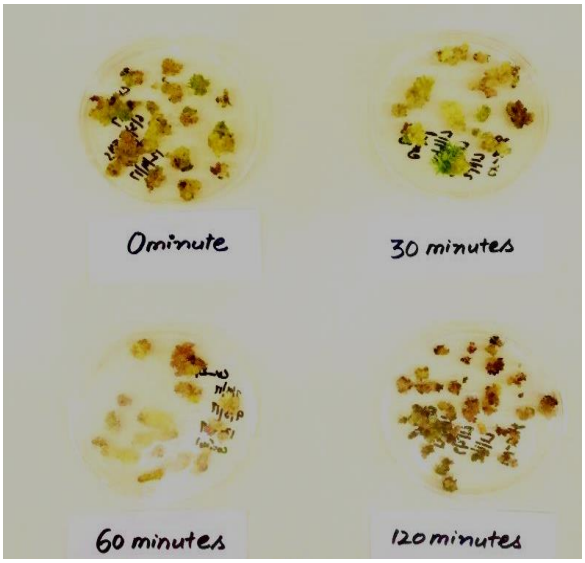

Fig 10. Different treatment times (0 minute, 30 minutes, 60 minutes and 120 minutes) of sugarcane *calli* with 0.5% EMS and their survival rate for determination of LD50.

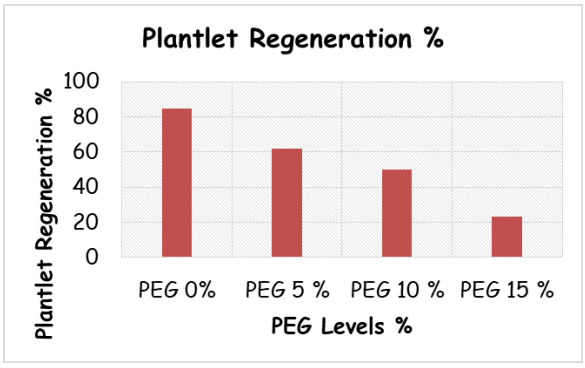

Fig 11. Optimization of different PEG levels for determination of LD50 of plant regeneration.

Exploitation of somaclonal variation in wheat for crop improvement

Different experiments were planned for tissue culture mediated crop improvement in wheat as detailed below.

The objective of the study was development of high yielding and disease free soma clones of wheat through callus cultures derived from immature /mature embryo. For this purpose ten R2 crosses and four wheat varieties Akber-2019, Dilkash-2020 and Subhani-2021 were used. Callus was induced on MS media with different doses of 2, 4 D (0, 2, 4 and 6 mg/l). Data was noted for different parameters of callus. For regeneration, Kinetin and NAA were used (Fig 12). When roots and shoots were properly developed, fifty somaclones of wheat were transferred to pots. The seed of wheat somaclones of different crosses and varieties were sown in field as R1 generation for testing against disease resistance and other morphological parameters.

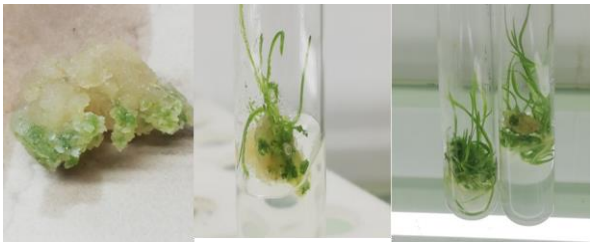

Fig. 12. Tissue culture optimization process of wheat showing callus induction, regeneration and rooting on different media in test tubes.

For optimization of protocol for maize mediated double haploid production in wheat, spikes of segregating generations (F_2 and F_3) were emasculated carefully that ovary were not damaged. Spike was covered with butter paper bag and placed all the spikes in incubation room for 2-3 days. Spikes were pollinated with maize genotypes (Malka & Pearl) and placed in pollination medium for three days then shifted into growth medium. Haploid seeds were developed after 10 to 12 days. Total 251 embryos were rescued under stereo microscope and cultured on half strength MS media. 41 haploid plants were treated with colchicine but no tillers produced which require additional efforts and colchicine treatment process needs to be further

optimized for obtaining better results (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.

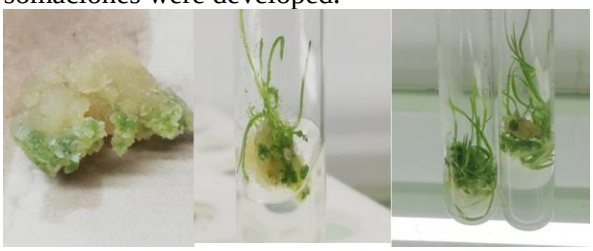

Fig. 13. Protocol optimization process of wheat showing callus induction, regeneration and rooting.

Protocol optimization of Teak plant micro-propagation

Shoot tips and buds were surface sterilized and cultured on MS medium supplemented with various cytokines and auxins combinations. Teak buds were sprouted and gained some success of shoot multiplication however rooting experiment is under process (Fig 13).

Fig 13. Teak micropropagated shoots showing potential for teak micropropagation.

In-vitro studies in sugarcane for inducing salt tolerance

Sugarcane advanced lines/varieties viz. CP-00-1101, S2016-SL-284, S2016-SL-306 were evaluated at different salt levels to see their inherited Callogenesis potential. Response to callogenesis among these sugar cane advance lines was evaluated at different salt levels to see their inherited callogenesis potential.

Frequency of callus initiation, fair to excellent, was recorded in CPF-1101 followed by SL-306 at 50 mm/L salt levels, while SL-284 at 100 mm/L. Callogenesis was suppressed at higher salt concentrations; however, variety/ line 1101 and 284 fairly showed enough callogenesis potential even at 150 and 200 m moles / L salt level (Fig 14).

Fig 14. Sugarcane callus induction and regeneration under salt stress.

D. SOIL BACTERIOLOGY & MICROBIOLOGY

Biofertilizers testing lab

Soil Bacteriology as “Biofertilizers Testing Lab” analyzed total **190** biofertilizers / biostimulants samples for registration or under FCO.

Table 5. List of samples biofertilizers samples tested in Biofertilizers testing lab during 2022-23.

Category	Sample analyzed	Fit
FCO	86	76
Registration	85	65
Advisory	19	13
Total	190	154

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

Field studies were conducted to determine the effect of PGPR and their metabolites on growth and yield of wheat and rice at ARS, Farooqabad and SSRI, Pindi Bhattian. Treatments included, control (T_1), PGPR (T_2), Kinetin @10^{-5} M (T_3), PGPR with Kinetin (T_4), PGPR Metabolites (Foliar spray) (T_5), PGPR Metabolites with Kinetin @10^{-5} M (Foliar spray) (T_6), PGPR + Metabolites of PGPR (Foliar spray) (T_2 + T_5) (T_7), PGPR + Metabolites of PGPR with Kinetin @10^{-5} M (Foliar spray) (T_2 + T_6) (T_8). Results of rice trials at ARS and SSRI, revealed that PGPR + Metabolites of PGPR with Kinetin produced the highest paddy yield i.e., 4620, 2839 kg ha^{-1}, respectively (Fig. 15). The same treatment produced the highest wheat grain yield i.e., 3570 and 3173 kg ha^{-1}, respectively, at both sites (Fig. 16).

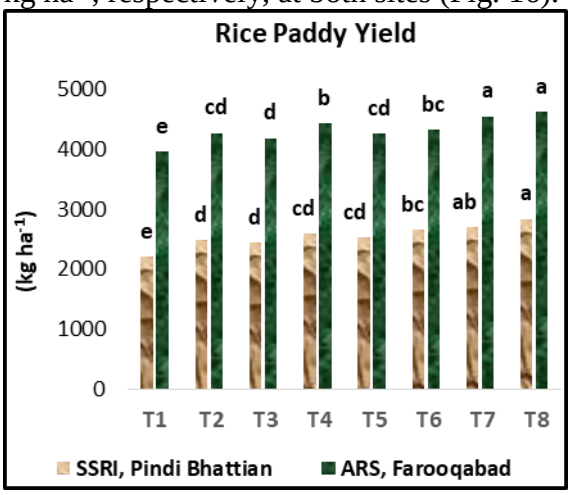

Fig. 15. Rice paddy yield at ARS, Farooqabad and SSRI, Pindi Bhattian.

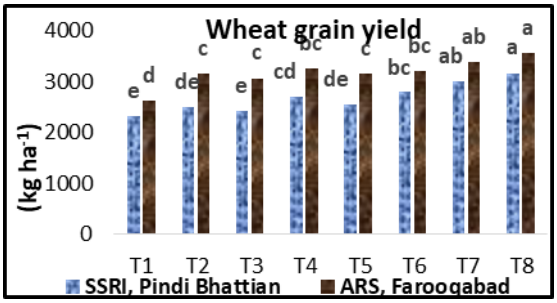

Fig. 16. Wheat grain yield at ARS, Farooqabad and SSRI, Pindi Bhattian.

Effect of some rhizospheric and endophytic bacteria for the growth and yield of cauliflower

A field experiment conducted to investigate the plant growth-promoting (PGP) abilities of bacterial strains isolated from cauliflower (rhizospheric and endophytic) with the aim to improve plant growth and yield through microbial application. The treatments were control (T_1) Rhizo-I (T_2), Rhizo-II (T_3), Endo-I (T_4), Endo-II (T_5) Rhizo-I +Endo-I (T_6), Rhizo-II+ Endo-II (T_7), Consortium (T_8). In this experiment soil having pH 8.0, EC 2.1, organic matter 0.70% and available P 9.6 mg kg^{-1} was used with recommended fertilizer. Results showed that significant increase in cauliflower yield was obtained from T_4 (38.4 t ha^{-1}) as compared to control (33.4 t ha^{-1}). (Fig. 17).

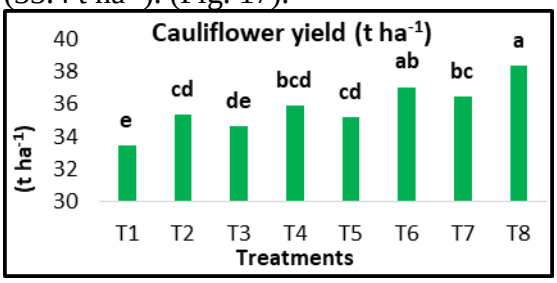

Fig. 17. Effect of rhizospheric and endophytic bacteria on yield of cauliflower

Effect of microbial inoculants and elemental Sulphur on growth and yield of canola

A field experiment on canola was conducted at Oilseed Research Institute, AARI, Faisalabad to evaluate the effect of microbial inoculants on growth and yield of canola. In

this experiment soil having pH 8.1, EC 1.1 dS m^{-1}, organic matter 0.76% and available P 9.7 mg kg^{-1} was used. Treatments were control (T_1), Elemental Sulphur (T_2), PGPR (T_3), *P. Indica* (T_4), PGPR+ Elemental Sulphur (T_5) and *P. Indica* + Elemental Sulphur (T_6). Results showed that maximum grain yield was obtained from T_6 treatment (2149 kg ha^{-1}) as compared to control (1850 kg ha^{-1}). (Fig. 18).

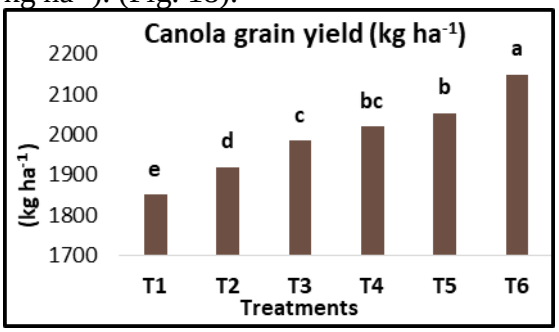

Fig. 18. Effect of elemental sulphur along with PGPR and P. indica on yield of canola.

Efficacy of microbial consortia in enhancing yield and quality of green chili

A field experiment was designed to assess the potential of plant growth promoting microbial consortia on growth and yield of green chili at Vegetable Research Institute Faisalabad. There were five treatments including control and four different consortia. These microbial consortia were applied by two methods i.e., root dipping and foliar application. In this experiment soil having pH 8.0, EC 2.7, Organic matter 0.73% and available P 9.6 mg kg^{-1} was used with recommended dose of fertilizer. Results showed that quantity and yield increased and maximum green chili yield was obtained from T_5 treatment where consortium was applied foliar (27.5 t ha^{-1}) as compared to control (21.5 t ha^{-1}). (Fig 19).

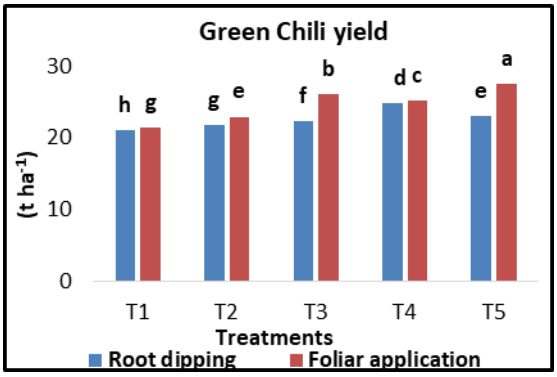

Fig. 19. Effect of different microbial consortium on yield of chili.

Growth and yield response of wheat and rice to PGPR and their metabolites having different characteristics features

Field study on rice was conducted at the Soil Chemistry Section, Faisalabad using normal soil having pH 8.21, EC 2.2 d Sm^{-1} and organic matter 0.59%. Field study on wheat was conducted at Soil Bacteriology Section, Faisalabad using normal soil having pH 8.1, EC 2.1 dS m^{-1} and organic matter 0.56%. Treatments were control (T_1), Auxin producing PGPR (T_2), Metabolites of T_2 (T_3), Siderophore producing PGPR (T_4), Metabolite of T_4 (T_5), Zinc solubilizing bacteria (T_6), Metabolites of T_6 (T_7). Result showed that T_6 produced maximum paddy yield i.e. 5000 kg ha^{-1} and wheat grain yield i.e. 3623 kg ha^{-1}. (Fig. 20-21).

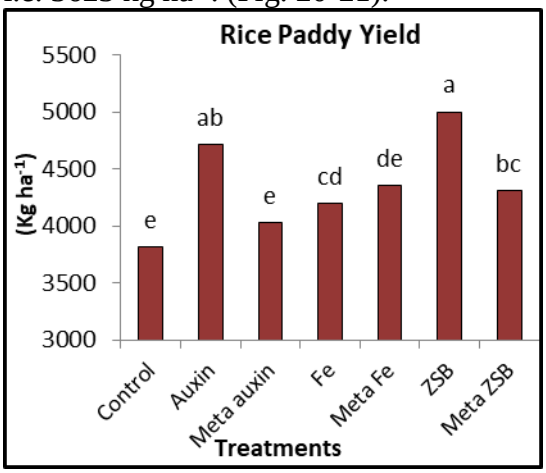

Fig. 20. Rice paddy yield at Soil Chemistry Section, Faisalabad.

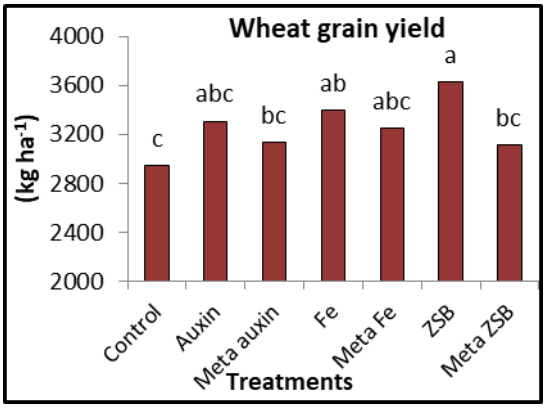

Fig. 21. Wheat grain yield at Soil Bacteriology Section, Faisalabad.

Comparison of different integrated techniques of iron application to improve yield and iron content of maize

Field experiments were conducted at Soil Chemistry Section, Faisalabad on maize to compare the application techniques to produce bio-fortified maize grain with improved crop production. The pre-sowing soil analyses of maize trial having texture sandy clay loam, pH 8.0, EC: 2.5 dS m^{-1}, available P 7.0 mg kg^{-1} and organic matter 0.76% with recommended dose of fertilizer @ 250-150-125 NPK kg ha^{-1}. Treatments were control RD NPK (T_1), RD + soil application Fe@ 10 kg ha^{-1} (T_2), RD + soil application Fe@ 20 kg ha^{-1} (T_3), RD + foliar application Fe@ 0.2% (T_4), RD+ Seed Inoculation with siderophore/SP (T_5), RD + soil application Fe @ 10 kg ha^{-1} + SP (T_6), RD + soil application Fe @ 20 kg ha^{-1} + SP (T_7), and RD + foliar application Fe @ 0.2% + SP (T_8). Results clearly indicated that inoculation with siderophore producing bacteria along with soil application of iron @ 20 kg ha^{-1} gave highest maize grain yield (6.60 t ha^{-1}) as compared to control (4.98 t ha^{-1}) (Fig. 22).

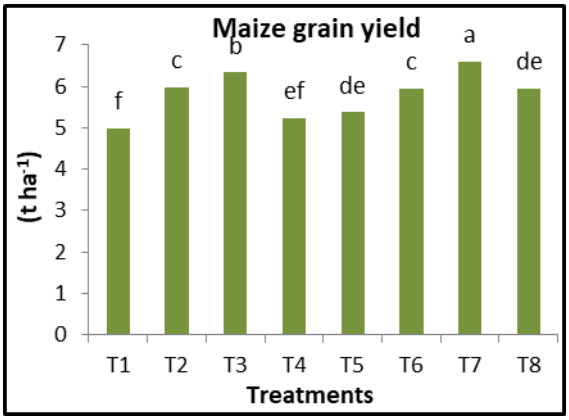

Fig. 22. Maize grain yield at Soil Chemistry Section, Faisalabad.

Precursor-inoculum interaction for improving growth and yield of canola and maize

The trial was conducted to assess the effect of PGPR along-with L-Tryptophan on canola and maize following RCBD layout with three repeats at Oil Seed Research Institute and Soil Bacteriology Section, Faisalabad. Treatments were control (T_1), PGPR-1 (T_2), PGPR-2 (T_3), L-Tryptophan @ 10^{-5} M (TRP) (T_4), PGPR-1 inoculation + L-Tryptophan @ 10^{-5}M (T_5) and PGPR-2 inoculation + L-TRP @ 10^{-5}M (T_6). Result showed that T_6 (PGPR-2 inoculation + TRP) showed maximum increase of canola grain yield i.e., 1950 kg ha^{-1} and fodder yield of maize i.e., 74.3 t ha^{-1}. (Fig. 23-24).

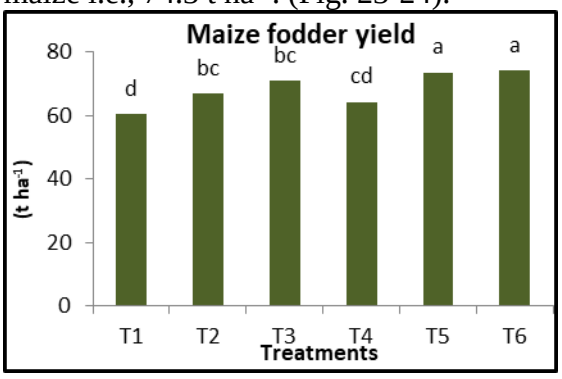

Fig. 23. Maize fodder yield at Soil Bacteriology Section, Faisalabad.

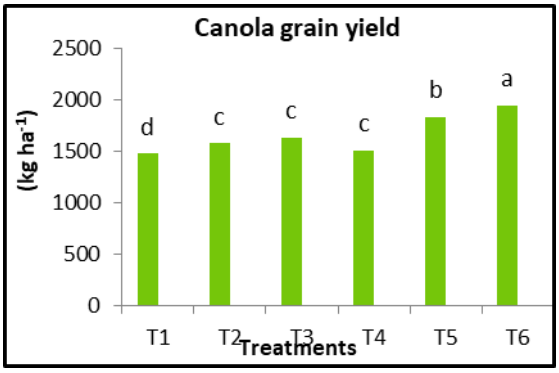

Fig. 24. Canola yield at Oilseed Research Institute, Faisalabad.

Plant microbe interaction for the growth and yield of oat fodder

This study was conducted to assess the plant microbe interaction for growth and yield of oat fodder at Fodder sub-station, Faisalabad. Soil is normal having pH 8.22, EC 2.1 dS m^{-1} and organic matter 0.59 following RCBD. Treatments were control (T_1), PGPR1 (*E. cloacae*) (T_2), (PGPR2 (*L. macroides*) (T_3), (Tryptamine (TRY) @ 10^{-5} M (Foliar spray) T_4), PGPR1+ PGPR2 (T_5), PGPR-1 + TRY @ 10^{-5} M (T_6), (PGPR-2 + TRY @ 10^{-5} M (T_7) and PGPR-1+ PGPR-2 + TRY @ 10^{-5} M (T_8). Result showed that T_8 which is PGPR-1+ PGPR-2 along with foliar spray of TRY gave highest fresh fodder yield 79.3 t ha^{-1}. (Fig. 25).

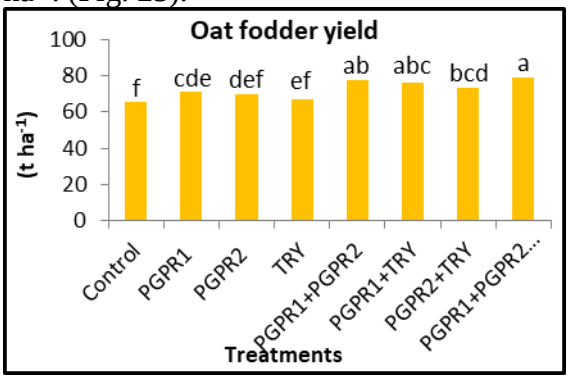

Fig. 25. Oat fodder yield at Fodder Sub-station, Faisalabad.

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

Seed treatment of efficient isolates of PGPR species were used as seed coating with or without Tryptamine (TRY) and L-Tryptophan (TRP). This trial was conducted with three repeats following RCBD at Soil Bacteriology Section and Agri. Biotech. Research Institute, Faisalabad on maize and wheat using normal soil having pH 8.0-8.3, EC 2.3-2.2 dS m^{-1} and organic matter 0.63-0.62%. Treatments were control (T_1), PGPR Inoculation (T_2), (TRY) @ 10^{-5} M Inoculation (T_3), TRP @ 10^{-5} M (T_4), PGPR + TRY @ 10^{-5} M (T_5) and PGPR + TRP @ 10^{-5} M (T_6). Result showed that PGPR + TRY produced maximum maize yield i.e. 54.6 t ha^{-1} and wheat yield 3353 kg ha^{-1} and that was significantly higher than control. (Fig. 26-27).

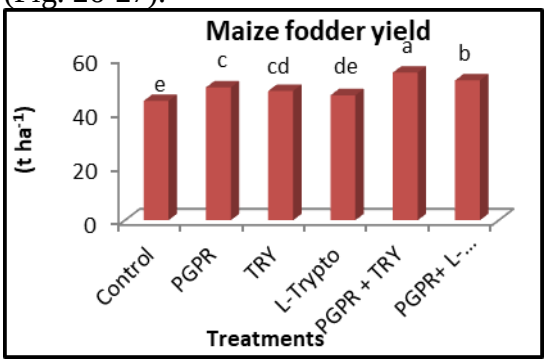

Fig. 26. Maize fodder yield at Soil Bacteriology Section, Faisalabad.

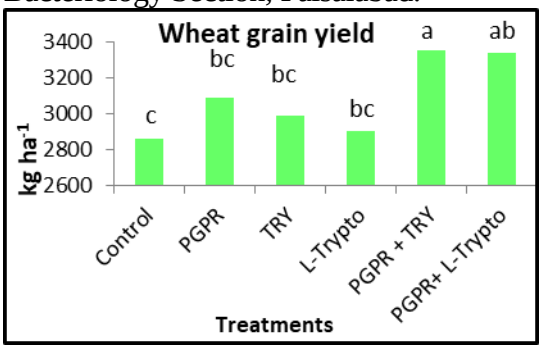

Fig. 27. Wheat grain yield at Agri. Biotech. Research Institute, Faisalabad.

Growth and yield response of maize to microbial inoculation and precursors of growth hormones

Efficient isolates of PGPR species available at Soil Bacteriology Section were used for inocula preparation. Seed treatment of respective PGPR bacteria were used as seed coating a while the precursors were applied to seed as seed soaking for one hour. Field

study was conducted at the Maize Research Station, Faisalabad on maize using normal soil having pH 8.0, EC 2.3 d Sm^{-1} and organic matter 0.63% following RCBD. Treatments were control (T_1), PGPR Inoculation (T_2), L-Tryptophan @ 10^{-5} M (T_3), L-Adenine @ 10^{-5} M (T_4), L-Methionine @ 10^{-5} M (T_5), PGPR Inoculation + L-Tryptophan @ 10^{-5} M (T_6), PGPR Inoculation + L-Adenine @ 10^{-5} M (T_7), PGPR Inoculation + L-Methionine @ 10^{-5} M (T_8). Maize crop was sown during Kharif 2021. Result showed that PGPR + Tryptamine showed significant increase in yield as compared to control in maize. The treatment T_6 produced maximum yield i.e. 5907 kg ha^{-1} that was significant increase as compared to control. (Fig. 28).

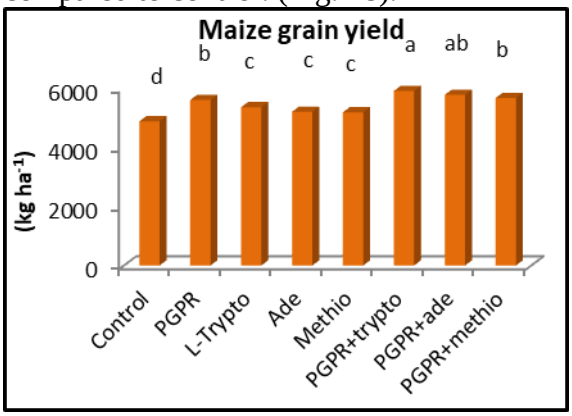

Fig. 28. Maize grain yield at Maize Research Station, Faisalabad.

Role of microbial consortium for decomposition of crop residues

To explore the potential of lignocellulolytic microbes in decomposition of crop residues, the existing isolates at Soil Bacteriology were tested for degrading ability of cellulose and lignin. The commercial product i.e., waste decomposer (WD) was also collected from market and analyzed. The bacterial isolates *Bacillus subtillis / Lysinibacillus macroides* and fungal isolate *Trichoderma* sp and their combination was prepared. There were six treatments i.e., control (T1), Bacterial application (T_2), Waste Decomposer (WD) (T_3), FYM (T_4) @ 2 t $acre^{-1}$, consortium (Bacteria + Fungi) (T_5), consortium + FYM (T_6). The treatments were tested with and without urea @1/2 bag $acre^{-1}$ and rotavating of field. Soil analysis was performed before and after harvesting of rice crop. Results indicated that microbial count and organic matter was increased while C:N ratio was decreased considerably. (Fig. 29)

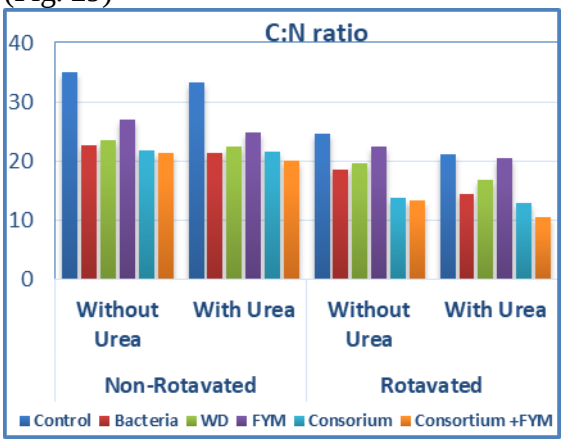

Fig. 29. The C:N ratio trend of crop residue decomposition at Soil Chemistry Section.

Development Projects:

Sr.	Proj. No.	Project Name
1.	PARB 20-206	Development of Resilient Cotton through Bridging Conventional and Biotechnological Approaches
2.	PARB 20-413	Genome-wide association studies to reveal the genetic architecture of grain yield under heat stress conditions in maize and DNA fingerprinting using SNPs for variety protection under Plant Breeders Rights Rules
3.	PARB 20-415	Molecular characterization of *Colletotrichum falcatum*: a responsible fungus of deadly sugarcane red rot disease
4.	PARB 20-421	Development and popularization of industrial quality durum wheat
5.	PARB 20-452	Development of Aflatoxin Biocontrol to minimize aflatoxin contamination in maize food chain and its commercialization
6.	PARB 22-325	Rice straw management by substrate stimulated microbial consortium to improve the soil and environmental health

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Miscellaneous:

1.	Radio Talks Delivered	02
2.	Visits of Delegations	30
3.	Training Seminars Delivered/Attended	40
4.	Class visits	25
5.	Internship Students	115

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